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Chromosomal repatterning in drosophila: *Drosophila nasuta nasuta* and *D. kohkoa*

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Abstract. Two three-break shifts (transpositions) are detected in a chromosome comparison between *D.n. nasuta* and *D. kohkoa*. Such aberrations are not reported in studies with chromosome comparisons in *Drosophila* species. The probable sequences are given to explain the occurrence of these transpositions.

Keywords. *Nastua* subgroup ; transpositions ; inversions.

1. Introduction

In *Drosophila*, phylogenetic relationships between species can be established by way of analysing the banding patterns in the salivary gland chromosomes. Perusal of the literature reveals that there is notable chromosomal differentiation in some groups of *Drosophila* (Bicudo 1973; Bock 1971; Brncic *et al* 1971; Hsu 1952; Kastritsis 1966; Stalker 1965; Stone *et al* 1966; Wasserman 1962a, b, c) while in others the banding sequences have apparently remained unaltered (Dobzhansky 1972). The latter is referred to as homosequential species.

The members of the *nasuta* subgroup of the *immigrans* group of the genus *Drosophila* have been studied to establish their evolutionary relationships. The members are, *D. nasuta nasuta*, *D.n. albomicana*, *D.n. kepulauan*, *D. kohkoa*, *D. pulaua*, *D. sui*, *D. nixifrons*, *D. pallidifrons*, *D. sulfurigaster sulfurigaster*, *D.s. neonasuta*, *D.s. bilimbata* and *D.s. albostrigata*. This is reported in detail by Rajasekarasetty *et al* (1980). The present paper deals with the chromosome relationship between *D.n. nasuta* and *D. kohkoa*. The nature of banding in *D. kohkoa* is studied in comparison with that of *D.n. nasuta* which is taken as the standard.

2. Materials and methods

As *D.n. nasuta* (of Coorg, Karnataka, India) and *D. kohkoa* (of Gulf of Thailand —University of Texas collection No. 3256-3 # 1) proved to be cross sterile (Rajasekarasetty *et al* 1980), a direct optical comparison of the banding pattern

of the salivary gland chromosomes of both the species were made. The procedure of Ranganath and Krishnamurthy (1975) was used to prepare the salivary gland chromosomes.

3. Results and discussion

The karyotype of both *D.n. nasuta* and *D. kohkoa* includes a pair of metacentric (chromosome 2), two pairs of acrocentrics (sex chromosome and chromosome 3) and a pair of dots (chromosome 4). The salivary gland chromosome complement of both the species thus includes four long arms representing two arms of chromosome 2 (2L and 2R), chromosome 3, X chromosome and a short arm of chromosome 4.

Comparison of banding patterns of the salivary gland chromosome of *D. kohkoa* with that of *D.n. nasuta* revealed that the X chromosome and chromosome 2 are homosequential but chromosome 3 of the former species differs from that of the latter by a paracentric inversion named NKo₁ and two three-break shifts (transpositions) named NKo-S₁ and NKo-S₂ (figures 1, 2).

Structural re-organization of the chromosomes during speciation involves either paracentric, pericentric inversions, duplications and/or deletions. Chromosomal differentiation due to these changes (aberrations) have been reported in different groups of *Drosophila*. The uniqueness of the present report is that, in addition to a paracentric inversion, two three-break shifts (transpositions) are also involved in the chromosomal repatterning in *D. kohkoa*. The existing chromosomal linearity due to transpositions in *D. kohkoa* could be explained by two successive inversions and the probable sequence of which is represented diagrammatically in figure 3.

Perusal of the literature reveals that the occurrence of such three-break shifts are very rare. Dobzhansky (cf. Patterson and Stone 1952) has expressed that there are no sure cases of three-break rearrangements in *Drosophila* species. Similarly White (1973) has opined that chromosomal repatterning due to transpositions is rare. As far as we know, this occurrence of transpositions is a maiden report of its kind for species comparisons in *Drosophila*.

1 ↓ a b c d e f g h i j k l m ↓ n —

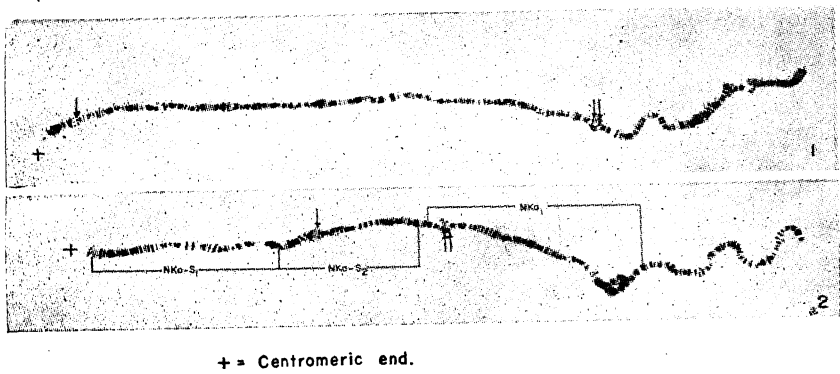
One inversion with two breaks (between—centromere and a, m and n)

2 ↓ m l k j i h g f ↓ e d c b a ↓ n — — —

Two inversions with three breaks (between—centromere m, f and e, a and l)

3 f g h i j k l m a b c d e n — — —

Figure 3. Diagrammatic representation of the possible/probable rearrangements to explain existing linearity of the chromosome 3 in *D. kohkoa* (when compared with chromosome 3 of *D.n. nasuta*, taken as standard).
(# Centromeric end).



Figures 1-2. 1. Chromosome 3 of *D. nasuta nasuta*. 2. Chromosome 3 of *D. kohkoa*.

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References

- Bicudo H E M C 1973 Chromosomal polymorphism in the *saltans* group of *Drosophila*. I. The *saltans* subgroup ; *Genetica* **44** 520-552
- Bock I R 1971 Intra and interspecific chromosomal inversions in the *Drosophila bipectinata* species complex ; *Chromosoma* **34** 206-229
- Brncic D, Nair P S and Wheeler M R 1971 Cytotaxonomic relationships within the *mesophragmatica* species group of *Drosophila* ; *Univ. Tex. Publ.* **7103** 1-16
- Dobzhansky Th 1972 Species of *Drosophila* ; *Science* **177** 664-669
- Hsu T C 1952 Chromosomal variation and evolution in the *virilis* group of *Drosophila* ; *Univ. Tex. Publ.* **5204** 35-72
- Kastritsis C D 1966 A comparative chromosome study in the incipient species of the *Drosophila paulistorum* complex ; *Chromosoma* **19** 208-222
- Patterson J T and Stone W S 1952 *Evolution in the genus Drosophila* (New York : The Macmillan Co.)
- Rajasekarasetty M R, Ramesh S R and Krishnamurthy N B 1980 Interspecific chromosomal variation among a few members of the *nasuta* subgroup (Genus : *Drosophila*) ; *Entomon.* **5** 1-12
- Ranganath H A and Krishnamurthy N B 1975 Chromosomal polymorphism in *Drosophila nasuta*. III. Inverted gene arrangements in South Indian populations ; *J. Hered.* **66** 90-96
- Stalker H D 1965 The salivary Chromosomes of *Drosophila micromelanica* and *Drosophila melanura* ; *Genetics* **51** 487-507
- Stone W S, Guest W C and Wilson F D 1960 The evolutionary implications of the cytological polymorphism and phylogeny of the *virilis* group of *Drosophila* ; *Proc. Natl. Acad. Sci. U.S.A.* **46** 350-361
- Wasserman M 1962a Cytological studies of the *repleta* group of the genus *Drosophila*. III. The *mercatorum* subgroup ; *Univ. Tex. Publ.* **6205** 63-71
- Wasserman M 1962b Cytological studies of the *repleta* group of genus *Drosophila*. IV. The *hydei* subgroup ; *Univ. Tex. Publ.* **6205** 73-83
- Wasserman M 1962c Cytological studies of the *repleta* group of the genus *Drosophila*. V. The *mulleri* subgroup ; *Univ. Tex. Publ.* **6205** 85-117
- White M J D 1973 *Animal cytology and evolution* (Third Edn.) Cambridge University Press

Acid phosphatase activity in tissues of *Notopterus notopterus* chronically exposed to phenolic compounds

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Abstract. Specimens of *Notopterus notopterus* were exposed to three sublethal concentrations (1/10th, 1/15th and 1/20th of 96 hr LC_{50}) of phenol (P), 2,4-dinitrophenol (DNP), pentachlorophenol (PCP), and their three combinations (PCP + DNP)/P (highly antagonistic), (DNP + P)/PCP (additive) and (P + DNP)/PCP (highly synergistic) for 15 and 30 days, and brain, liver, kidney and gills were taken out separately for determining acid phosphatase activity. In general, inhibition was maximum (89.32%) and highly significant ($P < 0.001$) in brain, and minimum (6.93%) and insignificant in kidney of fish exposed to 1/10th of (P + DNP)/PCP and P, respectively after 30 days. When P, DNP and PCP were used separately PCP exerted more inhibitory effects than DNP and P. However, significant stimulation ($P < 0.05$; $P < 0.01$) at 1/15th and 1/20th of P and DNP both after 15 and 30 days, and insignificant at 1/20th of (PCP + DNP)/P after 15 days was also observed in kidney.

Keywords. Acid phosphatases ; *Notopterus notopterus*, phenolic compounds.

1. Introduction

There is increasing concern today about environmental contamination with phenolic compounds such as phenol, 2,4-dinitrophenol and pentachlorophenol. These compounds are the non-specific pesticides (Rappe and Nilson 1972) used as herbicides, molluscicides and bactericides in industries, wood preservation and agriculture. As an antiseptic, phenol is also used for medicinal purposes. In spite of their extensive use, little attention is paid on their effects on metabolic activities of freshwater fish (Weinbach and Garbus 1969 ; Desai et al 1978 ; Dalela *et al* 1980 ; Verma *et al* 1980). Acid phosphatase is a hydrolytic enzyme which takes part in the dissolution of dead cells and as such is a good indicator of stress condition in the biological system (Gupta *et al* 1975 ; Verma *et al* 1980). This study was undertaken to evaluate the effects of sublethal concentrations of P, DNP, PCP and their three combinations—(PCP + DNP)/P (highly antagonistic), (DNP + P)/PCP (additive) and (P + DNP)/PCP (highly synergistic) (Verma *et al* 1981) on acid phosphatase activity (orthophosphoric monoester phosphohydrolase ; E.C. 3.1.3.2) in different tissues of a freshwater fish *Notopterus notopterus*.

2. Materials and methods

Specimens of *N. notopterus* (16 to 21 cm in length ; 35 to 60 g in weight) were collected from Kalinadi and adopted for two weeks to the laboratory conditions. The technical grades of phenol (C_6H_5OH), 2,4-dinitrophenol ($(NO_2)_2 C_6H_3OH$) and pentachlorophenol (sodium salt; C_6Cl_5ONa) manufactured by Thomas Baker and Co. (London), Thomas and Thomas (India) and Hopkins and William Ltd. (England), respectively were used. Stock solutions of 1.0 g/L were prepared separately and the desired concentrations of these chemicals were obtained, using the table 231 (3) of Standard Methods (1971).

Fifteen fish were transferred in each concentration (1/10th, 1/15th and 1/20th of 96 hr LC_{50}) of these chemicals and combinations kept in triplicate for 30 days (96 hr LC_{50} of P, DNP, PCP and (PCP + DNP)/P, (DNP + P)/PCP and (P + DNP)/PCP combinations being 12.53 mg/L, 1/34 mg/L, 0.083 mg/L, 24.00 mg/L, 0.083 mg/L and 0.0065 mg/L, respectively. During acclimatation and exposure periods, fish were fed once a day with chilled crustacean diet (containing cyclops and daphnia) to avoid the starvation effects (Alekseev and Uspenskaya 1974). Solutions were renewed after each 24 hr, to avoid the fouling by food and excretory matter. Controls were also set side by side for comparison.

Fish were sacrificed after 15 and 30 days, and brain, liver, kidney and gills were taken out and pooled separately in ice cold petridishes containing 0.25 M sucrose solution. Tissue homogenates (5%) were prepared separately using 0.25 M sucrose solution, with a Potter Elvehjem homogenizer. Homogenates were centrifuged at 900g under cold conditions ($5.0 \pm 1.0^\circ C$) and supernatants were used for enzyme study. Acid phosphatase activity was measured by the method of Shinowara *et al* (1942). The inorganic phosphate liberated was determined by Fiske and Subbarow (1925) method. Statistical significance of the difference between the control and experimental values was calculated by student's 't' test (Fisher 1950).

3. Results and discussion

Average values along with mean \pm S.E. of three observations for acid phosphatase activity in brain, liver, kidney and gills of control fishes, and per cent inhibition/stimulation in exposed fishes after 15 and 30 days are given in table 1. It is clear from the table that when fishes were exposed to P, DNP and PCP separately, greater inhibition was observed in fishes exposed to PCP and DNP as compared to fishes exposed to P. This is due to the replacement of hydrogen by chloro and nitro groups in PCP and DNP, respectively (Kopperman *et al* 1974). In general maximum (89.32%) and highly significant ($P < 0.001$) inhibition as observed in brain, and minimum and insignificant (6.93%) in kidney at 1/10 th concentration of (P + DNP)/PCP, respectively after 30 days. However, in kidney biphasic effects of P, and DNP were observed, i.e., inhibition in enzyme activity at higher concentrations and stimulation at lower concentrations. Stimulation was significant ($P < 0.05$) at 1/15th of P, 1/15th and 1/20th of DNP, and at $P < 0.01$ in 1/20th of P after 15 days, and 1/15th and 1/20th of P and DNP after 30 days while it was insignificant at 1/20th of (PCP + DNP)/P combination after 15 days. Inhibition in acid phosphatase in these tissues was in the order,

Table 1. Per cent alterations in acid phosphatase activity in certain tissues of *Notopterus notopterus* exposed to phenolic compounds after 15 and 30 days.

Tissues	Control enzyme activity*	Days	%Alterations in enzyme activity								
			P			DNP			PCP		
			1/10th	1/15th	1/20th	1/10th	1/15th	1/20th	1/10th	1/15th	1/20th
Brain	1.58±0.19		-40.28 ^b	-31.25 ^a	-25.54 ^a	-51.4 ^a	-39.0 ^b	-30.3 ^b	-61.0 ^a	-45.9 ^b	-40.6 ^b
Liver	68.18±2.95		-39.12 ^b	-30.27 ^a	-25.53 ^a	-50.1 ^a	-39.2 ^b	-32.6 ^b	-57.7 ^a	-48.2 ^b	-41.0 ^b
Kidney	3.95±0.41	15	-10.97	+20.91 ^a	+33.54 ^b	-28.6 ^a	+17.3 ^a	+25.6 ^a	-49.0 ^b	-40.8 ^b	-32.3 ^a
Gills	2.28±0.21		-40.59 ^b	-33.28 ^a	-28.56 ^a	-53.3 ^a	-40.6 ^b	-31.3 ^b	-60.3 ^b	-47.2 ^b	-41.0 ^b
Brain	1.58±0.26		-53.17 ^a	-39.28 ^b	-33.89 ^b	-60.3 ^a	-49.5 ^b	-40.8 ^b	-79.0 ^a	-60.8 ^a	-52.6 ^a
Liver	68.18±2.95	30	-50.28 ^b	-42.16 ^b	-33.59 ^a	-62.9 ^a	-48.2 ^b	-40.6 ^b	-72.7 ^a	-60.5 ^a	-51.2 ^a
Kidney	3.95±0.41		-6.93	+28.21 ^b	+34.56 ^b	-30.3 ^b	+26.0 ^b	+34.3 ^b	-62.1 ^a	-49.1 ^b	-40.6 ^b
Gills	2.28±0.25		-50.94 ^a	-43.26 ^a	-40.54 ^b	-60.1 ^a	-48.3 ^b	-37.5 ^b	-71.1 ^a	-58.2 ^a	-52.2 ^a
<hr/>											
			(PCP+DNP)/P			(DNP+P)/PCP			(P+DNP)/PCP		
Brain	1.58±0.25		-38.2 ^b	-27.5 ^a	-24.1 ^a	-64.5 ^b	-48.1 ^b	-40.8 ^a	-65.6 ^a	-46.9 ^b	-39.9 ^a
Liver	68.15±2.95		-43.1 ^a	-34.6 ^a	-30.2 ^a	-58.8 ^a	-43.1 ^b	-34.2 ^b	-59.3 ^b	-45.2 ^b	-38.2 ^b
Kidney	3.91±0.48	15	-15.3	-9.8	+10.5	-45.8 ^b	-38.0 ^a	-30.9 ^a	-47.9 ^b	-33.3 ^a	-26.4 ^a
Gills	2.26±0.41		-33.2	-26.8	-22.5	-59.3 ^b	-45.9 ^b	-40.1 ^a	-53.9 ^b	-40.1 ^b	-31.7 ^a
Brain	1.56±0.13		-47.5 ^a	-36.8 ^b	-30.1 ^b	-81.2 ^a	-63.7 ^b	-50.5 ^b	-89.3 ^a	-68.7 ^a	-52.1 ^b
Liver	68.14±2.95		-52.2 ^a	-33.1 ^b	-37.2 ^b	-75.2 ^a	-58.2 ^a	-49.2 ^a	-80.2 ^a	-60.1 ^a	-47.7 ^a
Kidney	3.91±0.40	30	-21.5 ^a	-13.3	-8.9	-60.7 ^b	-48.3 ^a	-41.5 ^a	-62.0 ^b	-40.7 ^a	-35.5 ^a
Gills	2.25±0.16		-44.2 ^a	-35.7 ^a	-30.2	-74.6 ^a	-59.0 ^b	-50.0 ^b	-70.2 ^a	-50.2 ^b	-42.5 ^a

* Enzyme activity is expressed as 'μg inorganic phosphate liberated/min.

* Enzyme activity is expressed as μg inorganic phosphate liberated/mg tissue/hr.Values are mean \pm S.E. of three observations.

+ indicate per cent stimulation ; - indicate per cent inhibition.

Per cent alteration is statistically significant at a : $P < 0.05$; b : $P < 0.01$; c : $P < 0.001$.

brain > liver > gills > kidney except after 30 days in DNP and after 15 and 30 days in (PCP + DNP)/P where inhibition was in the order, liver > brain > gills < kidney, and after 30 days in P where sequence of inhibition was in the order, brain > gills > liver > kidney.

Phenols enter in blood circulation of fish through gills and skin, and get distributed into different tissues where they affect normal metabolism (Mitrovic *et al* 1968). Dalela *et al* (1980) also studied the effect of sublethal concentrations of P and PCP on hepatic acid and alkaline phosphatases and observed significant inhibition. Synergistic effects of P and DNP on acid and alkaline phosphatases were also observed by Verma *et al* (1980). Authors in this investigation observed that these compounds in combinations showed no definite pattern of toxicity (i.e., inhibition/stimulation in enzyme activity) as they showed separately. At 1/20th, in brain after 15 and 30 days, at 1/10th, 1/15th and 1/20th in liver after 15 days, at 1/15th and 1/20th in liver after 30 days, and at 1/10th in kidney after 15 days, the per cent inhibition was not significantly different in fish exposed to PCP, (DNP + P)/PCP and (P + DNP)/PCP combinations. At 1/15th and 1/20th in kidney after 15 and 30 days, at 1/10th, 1/15th and 1/20th in gills after 15 days, and at 1/15th and 1/20th in gills after 30 days inhibition in fish exposed to (P + DNP)/PCP was significantly lesser than in fish exposed to PCP alone and to (DNP + P)/PCP combination. In fish exposed to (PCP + DNP)/P, inhibition in brain and gills was significantly less as compared to the fish exposed to phenol, in liver inhibition was not significantly different and in kidney significant stimulation ($P < 0.05$; $P < 0.01$) was there at 1/15th and 1/20th of P both after 15 and 30 days while in (PCP + DNP)/P insignificant stimulation was observed at 1/20th only after 15 days.

Loomis and Lipmann (1948) and Simon (1953) after DNP exposure, and Yap *et al* (1975) and Desaijah (1978) after PCP exposure, pointed out that uncoupling of oxidative phosphorylation is the main cause for inhibition of phosphatases. Uncoupling of oxidative phosphorylation was also pointed out by Dalela *et al* (1980) and Verma *et al* (1980) for the inhibition of acid and alkaline phosphatases. Simon (1953) stated that concentrations higher than those needed to prevent oxidative phosphorylation injured the mitochondrial system so greatly as to block the action of enzymes concerned with oxidative metabolism. Action of uncouplers of oxidative phosphorylation has been pointed out on the basis of chemical (Pressman 1963) and chemi-osmotic (Mitchell 1961) interactions. According to (Pressman 1963), uncouplers promote the conductivity of protons within mitochondrial membranes and subsequently prevent the formation of a gradient across the membrane. According to Mitchell (1961), uncouplers promote the splitting of an energy rich intermediate compound prior to ATP production. Weinbach and Garbus (1969) suggested that uncouplers traverse through lipoprotein layer of mitochondrial membrane and interact with protein groups that then undergo structural changes. It is generally assumed that major changes in mitochondrial function are reflected in morphological alterations and that normal mitochondrial profiles are dependent on the continuing supply of energy rich intermediates produced by oxidative phosphorylation. Weinbach and Garbus (1969) indicated that these uncouplers bind tightly with mitochondrial proteins which are involved in amino acid metabolism. However, authors of this investigation, assumed that all these interactions and processes held simultaneously when

sh were exposed to these chemicals and their combinations, causing the uncoupling of phosphorylation and finally affect the activity of phosphatases. Mixing of chemicals enhances toxicity (synergism) in some cases and decreases (antagonism) in other cases but the actual mechanism of combination effects on acid phosphatase activity is not well-known.

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References

- Alekseev V A and Uspenskaya N E 1974 Toxicological characteristics of acute phenol poisoning of some fresh water worms ; *Gidrobiol. Zh.* 10 48-55
- Dalela R C, Rani S and Verma S R 1980 Physiological stress induced by sublethal concentrations of phenol and pentachlorophenol in *Notopterus notopterus* : Hepatic acid and alkaline phosphatases and succinic dehydrogenase ; *Environ. Pollut.* 21 3-8
- Desaiah D 1978 Effect of pentachlorophenol on the ATPases in rat tissues ; *Pentachlorophenol* 12 277-283
- Fisher R A 1950 (Statistical methods for research workers ; 11th ed. (London : Oliver and Boyd).
- Fiske G H and Subbarow K 1925 The colorimetric estimation of phosphorus ; *J. Biol. Chem.* 66 375-381
- Gupta P K, Dhar U and Bawa S R 1975 Effect of malathion and radiation separately and jointly upon rat enzymes *in vivo* ; *Environ. Physiol. Biochem.* 5 49-53
- Kopperman H L, Carlson R M and Caple R 1974 Aqueous chlorination and ozonation studies. Structure-toxicity correlations of phenolic compounds to *Daphnia magna* ; *Chem.Biol. Interaction* 9 245-251
- Loomis W F and Lipmann F 1948 Reversible inhibition of the coupling between phosphorylation and oxidation ; *J. Biol. Chem.* 173 807-814
- Mitchell P 1961 Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism ; *Nature (London)* 191 144-148
- Mitrovic V V, Brown V M, Shurben D G and Barryman M H 1968 Some pathological effect of subacute and acute poisoning of rainbow trout by phenol in hard water ; *Water Res.* 2 249-254
- Pressman B C 1963 In, Energy linked functions of mitochondria (ed.) B Chance (New York : Academic Press) pp 188-191
- Rappe C and Nilsson C A 1972 An artifact in the gas chromatographic determination of impurities in pentachlorophenol ; *J. Chromatogr.* 67 247-253
- Shinowara G Y, Johns L M and Reinhart H L 1942 The estimation of serum inorganic phosphate and acid and alkaline phosphatase activity ; *J. Biol. Chem.* 142 921-928
- Simon E W 1953 Mechanism of dinitrophenol toxicity ; *Biol. Rev. Cambridge Philos. Soc.* 28 453-479
- Standard methods for the examination of water and waste water 1971 13th ed. Am. Publ. Hlth. Assoc. Inc. New York, N.Y.
- Verma S R, Rani S and Dalela R C 1980 Effects of phenol and dinitrophenol on acid and alkaline phosphatases in tissues of a fish (*Notopterus notopterus*) ; *Arch. Environ. Contam. Toxicol.* 9 451-459

- Verma S R, Rani S and Dalela R C 1981 Synergism, antagonism and additivity of phenol, pentachlorophenol and dinitrophenol to a fish (*Notopterus notopterus*); *Arch. Environ. Contam. Toxicol.* **10** 365-371
- Weinbach E C and Garbus J 1969 Mechanism of action of reagents that uncouple oxidative phosphorylation; *Nature (London)* **221** 1016-1018
- Yap H M, Desai D, Cutkomp L K and Koch R B 1975 *In vitro* inhibition of fish brain ATPase activity by cyclodiene insecticides and related compounds; *Bull. Environ. Contam. Toxicol.* **14** 163-167

Differences in home ranges of rhesus monkey (*Macaca mulatta*) groups living in three ecological habitats

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Abstract. Field observations were carried out on rhesus monkeys living in Asarori Forest, Chakia Forest, and temples. Data on group size, group composition and socionomic sex-ratios were obtained. An average home range size in these three habitats was found to be 5.18 km², 1.52 km² and 0.017 km² respectively. A positive correlation was found between group size and home range size in the Asarori Forest. Core areas were absent inside the home ranges in Chakia Forest. The average core area in other 2 habitats was 0.48 km² and 0.009 km² in Asarori Forest and temples respectively. The variability in home ranges and core areas is analysed in terms of differences in ecological conditions.

Keywords. Home range ; phylogenetic adaptation ; adaptive modification ; rhesus monkey.

1. Introduction

In our earlier studies (Pirta and Singh 1978, 1980) we have emphasised the phylogenetic adaptiveness of home ranges in rhesus monkeys. The nature of a phylogenetically adaptive behavioural system varies from an extremely environmentally labile to a highly environmentally stable one (Lorenz 1965). The reviews by Clutton-Brock and Harvey (1977) and Southwick and Siddiqi (1974) indicate that home range size is an environmentally labile behavioural system and varies greatly both within and between the species of non-human primates. Although the home ranges of Hanuman langur (Vogel 1977) and bonnet monkey (Rahman and Parthasarthy 1978) have been studied in various ecological habitats in India, such comparative data on rhesus monkey are lacking. Such information helps in understanding the adaptive modifications going on in the behaviour of a species. They result from the interaction of phylogenetically acquired blueprints and the environment. In the present study our observations on the home ranges of rhesus monkey inhabiting 3 natural environmental conditions are reported.

2. Method

During the exploratory phase we became thoroughly acquainted with the geographical features of all 3 habitats. Our main emphasis was to record the loca-

tion of a monkey group as accurately as possible on a map, whenever and wherever it was encountered. A group was followed from a few minutes to several hours at a stretch on an observation day. Occasionally, a group was followed from dawn to dusk and during late evening and early morning hours. The period of study and time devoted to observations in different habitats are given in table 1. Behavioural observations were started after the monkeys became acquainted with the observer. We observed the monkeys by standing at the periphery of the group. All recordings were made on notebooks and maps *ad lib*. The main variables measured are given in table 2. However, qualitative notes of the ecological characteristics of a habitat and behaviour of monkeys were also taken.

3. Study areas

3.1. Asarori forest

The study site (32 km²) included blocks of Laldhang, Chandrabani, Asarori, Mahobawala and Mohamadpur (compartments 1, 2 and 3 only), which form a major portion of Asarori forest range in the Western division of Dehra Dun forest (figure 1). The Asarori forest is on the northern slope of the Siwalik Hills, with elevations ranging from 425 m at the valley floor to 950 m at the Siwalik crest. Detailed description of the Asarori forest has been reported by Lindburg (1971). The major area of the forest part studied was covered by *Shorea robusta* which was interspersed with other tree species, grassland and eroded stream beds or raos.

Table 1. Period of study and groups observed in different habitats.

Habitat	Area explored	Year	Days	Groups
Asarori Forest	32 km ²	Jan. 1974 to Dec. 75	400	13
Chakia Forest	24 km ²	Aug. 1977 to July 78	70	3
Urban area	40 km ²	do	150	2

Table 2. Sampling variables and their measures

Variables	Measures
1. Group size	Number of individuals which regularly associate together and share a common home range.
2. Group composition	Number of individuals in each age-sex class, i.e. adult males, adult females, juveniles and infants.
3. Home range size	Total area (km ²) over which the group was seen moving and foraging during one year period.
4. Core area size	Area (km ²) within the home range most frequently used for night resting.

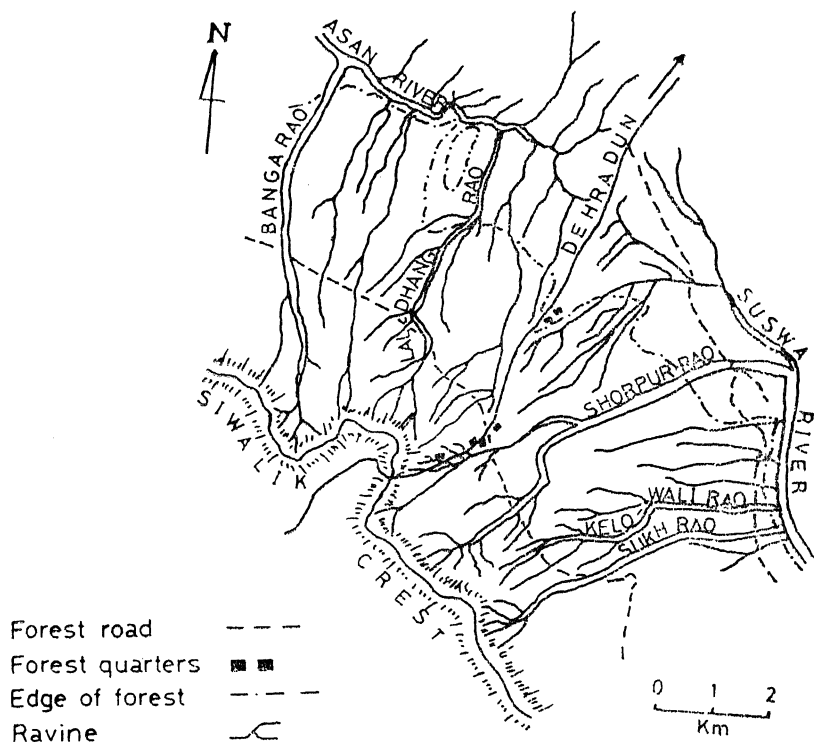


Figure 1. Map showing the principal features of forest habitat in Asarori.

3.2. Chakia forest

The study site (24 km²) was an isolated part of Chakia forest range of Varanasi division, and included four blocks : Sapahi, Sherpur, Amlahwa and Garhar (figure 2). It was mainly a scrub forest covered by interspersed trees of *Tamarindus indica*, *Azadirachta indica*, *Mangifera indica*, *Syzygium cumini*, *Semecarpus anacardium*, *Tectona grandis*, *Bombax malabaricum*, *Butea monosperma* and young plantations of bamboo and acacia. Shrubs of *Smilax indica*, *Carissa spinarum*, *Abrus precatorius* and *Ziziphus mauritiana* formed a thick vegetation along the ravines. The whole area was surrounded by cultivated land. In the southern part were 2 hillocks while the remaining area was plain but interspersed with deep ravines. The river Karamanasha flows in the middle of this forest part from south to north, accompanied by its 2 canals.

3.3. Urban area

The Varanasi city and its surrounding area, covering approximately 40 km², was explored for urban monkey population. Finally, two temples inside the city, each with a resident monkey group were selected for long term observations.

3.3a. *Sankat Mochan temple* : This temple was surrounded by a boundary wall and covered approximately 1.2 hectares. On both sides of the main temple

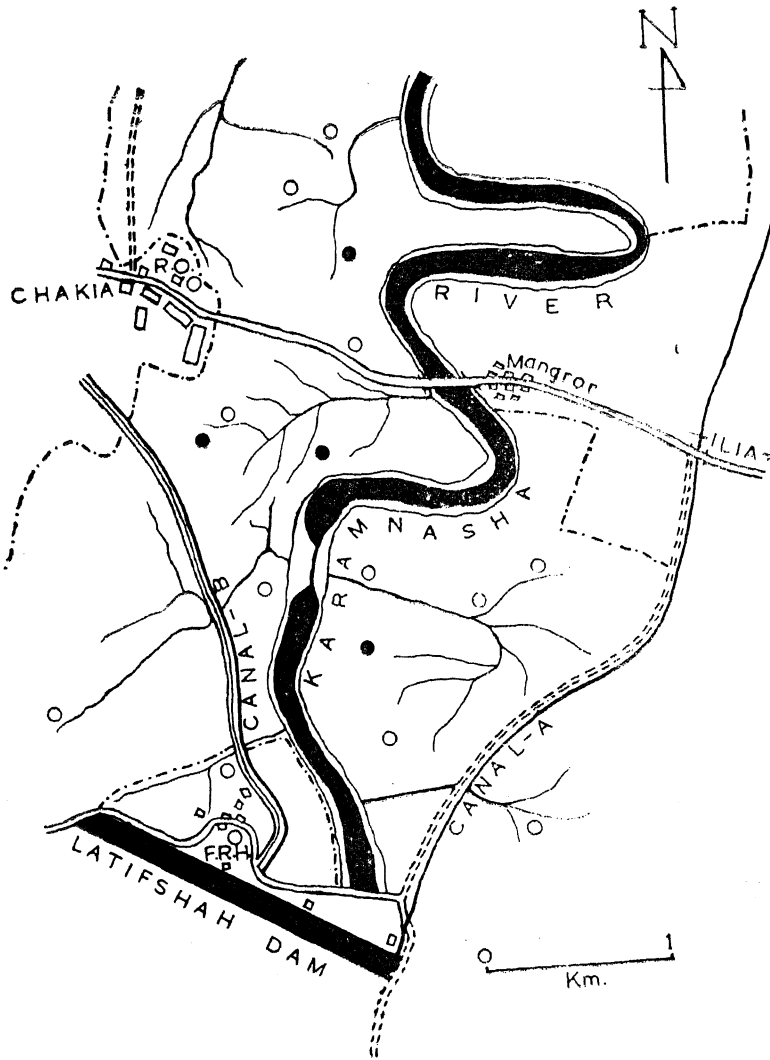


Figure 2. Map showing the principal features of forest habitat at Chakia. Black circles show rhesus groups and empty circles langur groups.

building was a thick vegetation of trees and shrubs. The tree species in this temple included *Ficus religiosa*, *Ficus bengalensis*, *Azadirachta indica*, *Phyllanthus emblica*, *Semecarpus anacardium* etc. There were shrubs of *Carissa spinarum*, *Smilax indica* and *Ziziphus mauritiana*. Various kinds of vegetables and grasses were also grown in the temple and its adjacent gardens. The Sankat Mochan temple is the temple of the monkey god Hanuman. People visited this temple specifically on Tuesdays and Saturdays to feed the monkeys. Other features of the temple are shown in figure 3.

3.3b. Durga temple : This temple was in the midst of buildings and covered approximately 0.6 hectares. On one side of it was a big pond. Except for a few trees in the compounds of adjacent buildings there was no vegetation in

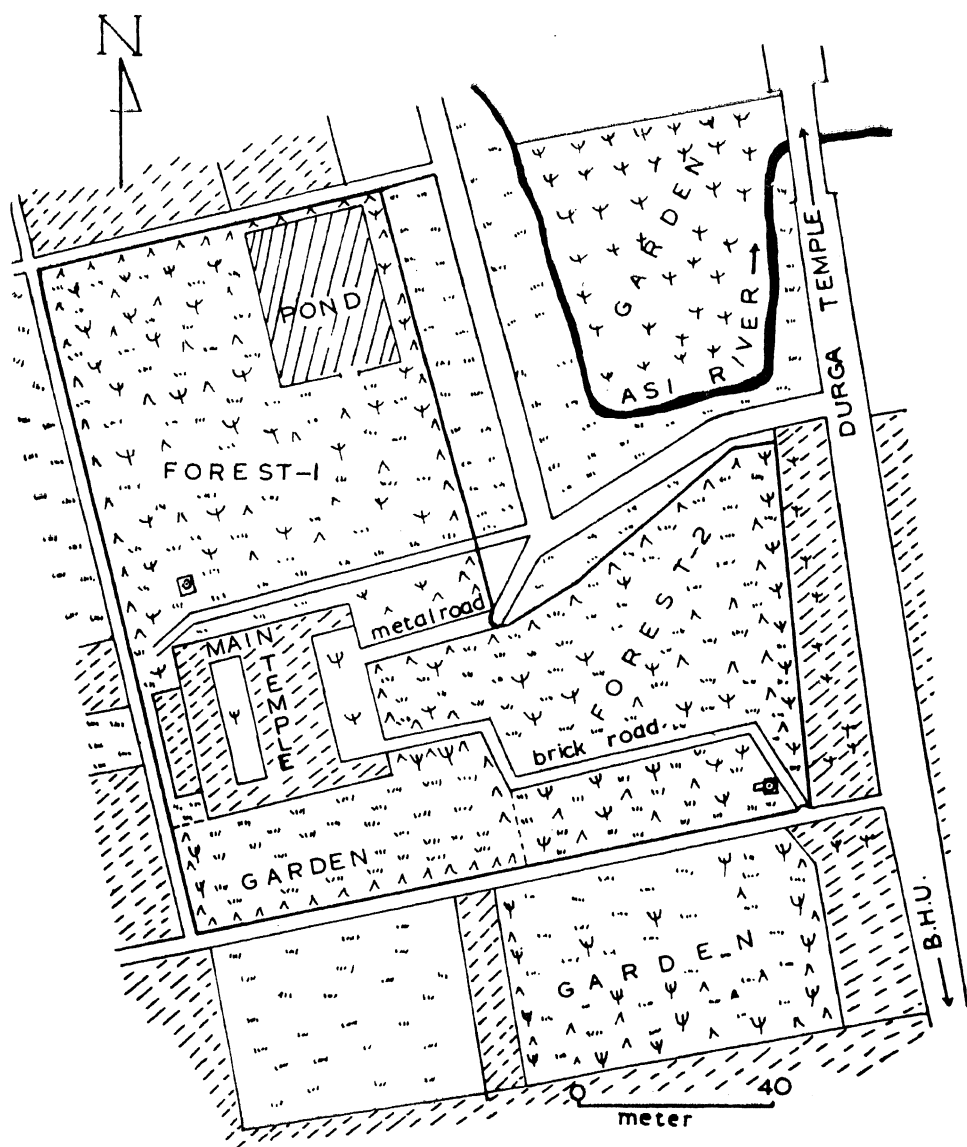


Figure 3. Principal features of Sankat Mochan temple.

Durga temple. There was less open space for monkeys in this temple in comparison to the Sankat Mochan temple. Because the Durga temple was located just on the side of the main road, the monkeys of this temple had more contact with human beings than those of the Sankat Mochan temple. Other habitat features of Durga temple are shown in figure 4.

A comparison of Asarori forest, Chakia forest and temple habitats is given in table 3.

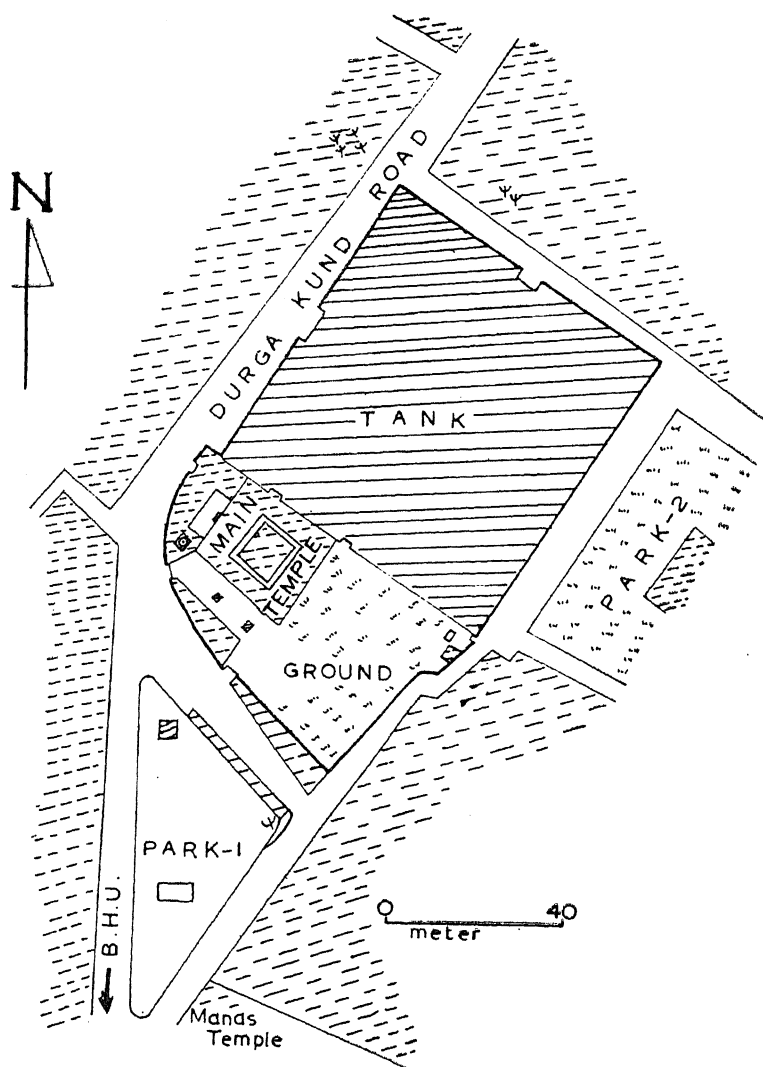


Figure 4. Principal feature of Durga temple.

4. Observations

Rhesus monkeys live in groups which comprise of adult males, adult females, juveniles and infants. A group occupies a circumscribed area of a particular niche, the home range.

4.1. Group size

In all 13 bisexual groups, 3 temporary all-male associations and 2 solitary males were observed in Asarori forest. A total of 598 monkeys lived in an area of 32 km². The number of monkeys in bisexual groups varied from 11 to 127 with an average group size of 45 (table 4).

Table 3. Comparison of the three habitats.

Characteristics	Temples, Varanasi	Chakia forest, Varanasi	Asarori forest, Dehra Dun
Temperature	7° C–41° C	7° C–41° C	0° C–40° C
Annual rainfall	1088 mm	1088 mm	1600 mm
Vegetation	A few trees in Sankat Mochan temple	Scrub forest	Moist Deciduous forest
Human influence	High	Medium	Very less
Other wild mammals	Jackal (in Sankat Mochan temple)	Jackal, pig, leopard (?)	Jackal, pig, deer species, antelopes, elephants, leopard
Sleeping trees	Roof (Durga temple) and trees (Sankat Mochan temple)	Few trees	Numerous trees
Water sources	Many	Many	Few
Food sources	Good, localised	Poor, scattered	Good, scattered
Predators	Man, dog, hawk	Man, dog, hawk, leopard (?)	Man, dog, weasel, hawk, leopard
Other primates	None	Langur	Langur

Table 4. Numerical data on group size, group composition, home range size and core area of rhesus groups in Asarori forest.

Group	Group size	Group composition *				Home range (km ²)	Core area (km ²)	Socionomic sex-ratio (MM : FF)
		MM	FF	JJ	II			
G 1	30	3	8	12	7	5.06	0.56	1:2.66
G 2	127	11	35	61	20	14.06	1.81	1:3.18
G 3	11	1	3	4	3	1.12	0.04	1:3.00
G 4	77	8	25	29	15	9.56	0.88	1:3.12
G 5	70	6	21	33	10	11.25	1.13	1:3.50
G 6	37	2	10	19	6	1.75	0.07	1:5.00
G 7	33	2	9	15	7	2.75	0.14	1:4.50
G 8	37	3	9	18	7	3.93	0.32	1:3.00
G 9	28	3	9	12	4	3.93	0.22	1:3.00
G 10	32	4	10	12	6	2.25	0.11	1:2.50
G 11	37	3	9	18	7	5.06	0.56	1:3.00
G 12	37	3	10	17	7	3.93	0.32	1:3.33
G 13	28	2	7	13	6	2.81	0.14	1:3.50
Mean	44.92	3.92	12.69	20.23	8.07	5.18	0.48	1:3.33
S.E.M.	± 8.36	± 0.78	± 2.45	± 3.97	± 1.28	± 1.09	± 0.20	

* Based on census in June-July 1974 ; MM—adult males ; FF—adult females ; JJ—juveniles ; II—infants.

In the Chakia forest, 5 bisexual groups and 1 isolated male lived in an area of 24 km². Three groups counted ranged from 27 to 38 ; an average group had 31.6 individuals (table 5).

In the city of Varanasi 9 bisexual groups were located in an area of 40 km² approximately. Two temple groups were counted, providing an average group size of 98.5 (table 6).

Table 5. Numerical data on group size, group composition and home range size of rhesus groups in Chakia forest.

Group	Group size	Group Composition *				Home range km ²	Socionomic sex-ratio (MM : FF)
		MM	FF	JJ	II		
Group 1	38	3	17	12	6	3.00	1:5.66
Group 2	27	2	11	7	7	0.56	1:5.50
Group 3	30	3	17	5	5	1.00	1:5.66
Mean	31.66	2.66	15.00	8.00	6.00	1.52	1:5.60
S.E.M.	± 3.31	± 0.35	± 2.00	± 2.08	± 0.57	± 0.75	

* Based on census in December 1977 ; MM—adult males ; FF—adult females ; JJ—juveniles ; II—infants.

Table 6. Numerical data on group size, group composition, home range size and core area of temple monkeys.

Group	Group size	Group composition *				Home range km ²	Core area km ²	Socionomic sex-ratio (MM:FF)
		MM	FF	JJ	II			
Sankat Mochan temple group	129	13	40	46	30	0.020	0.012	1:3.07
Durga temple group	68	7	20	28	13	0.015	0.006	1:2.85
Mean	98.50	10.00	30.00	37.00	21.50	0.017	0.009	1:2.96
S.E.M.	± 30.50	± 3.00	± 10.00	± 9.00	± 8.50	0	0	

* Based on census in December 1977 ; MM—adult males ; FF—adult females ; JJ—juveniles ; II—infants.

4.2. Group composition

An average group in Asarori comprised of 3.9 adult males, 12.7 adult females, 20.2 juveniles and 8 infants. In Chakia forest an average group size comprised of 2.6 adult males, 15 adult females, 8 juveniles and 6 infants. There were 10 adult males, 30 adult females, 37 juveniles and 21.5 infants in an average temple group. Socioeconomic sex-ratios (adult males : adult females) in Asarori, Chakia forest and temples were 1 : 3, 1 : 5 and 1 : 3 respectively.

4.3. Home range size

An average home range size of 13 bisexual groups in Asarori forest was 5.18 km². The home range remained the same for 2 years except for some minor changes in the case of some groups. There was extensive overlapping of home ranges among 13 groups of Asarori forest. A group shared its home range with at least 4 other groups (figure 5). The largest group (G2) shared the home range of 10 groups. A relationship was found between the group size and home range size. As the number of individuals increased the size of the home range also increased (product-moment coefficient of correlation, $r = 0.934$; $df = 11$; $p < .01$).

In Chakia forest the average home range size was 1.52 km² for the 3 rhesus groups. The overlapping of home ranges was less in the Chakia forest when compared to the Asarori forest. The home ranges in Chakia forest were also smaller in size (figure 6).

The temple group lived in an average home range of 0.017 km². There was no overlapping among the home ranges of Durga temple group and Sankas Mochan temple group (figures 7 and 8). Whenever another group was seen on the periphery of Durga temple group home range, the latter group immediately chased the former group away.

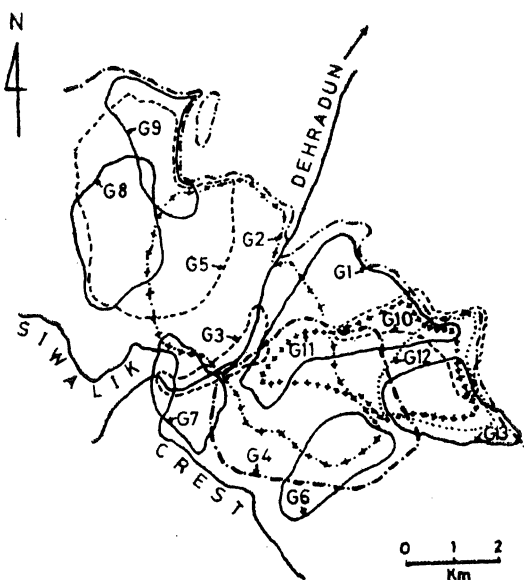


Figure 5. Home ranges of rhesus monkeys occupying the Asarori forest.

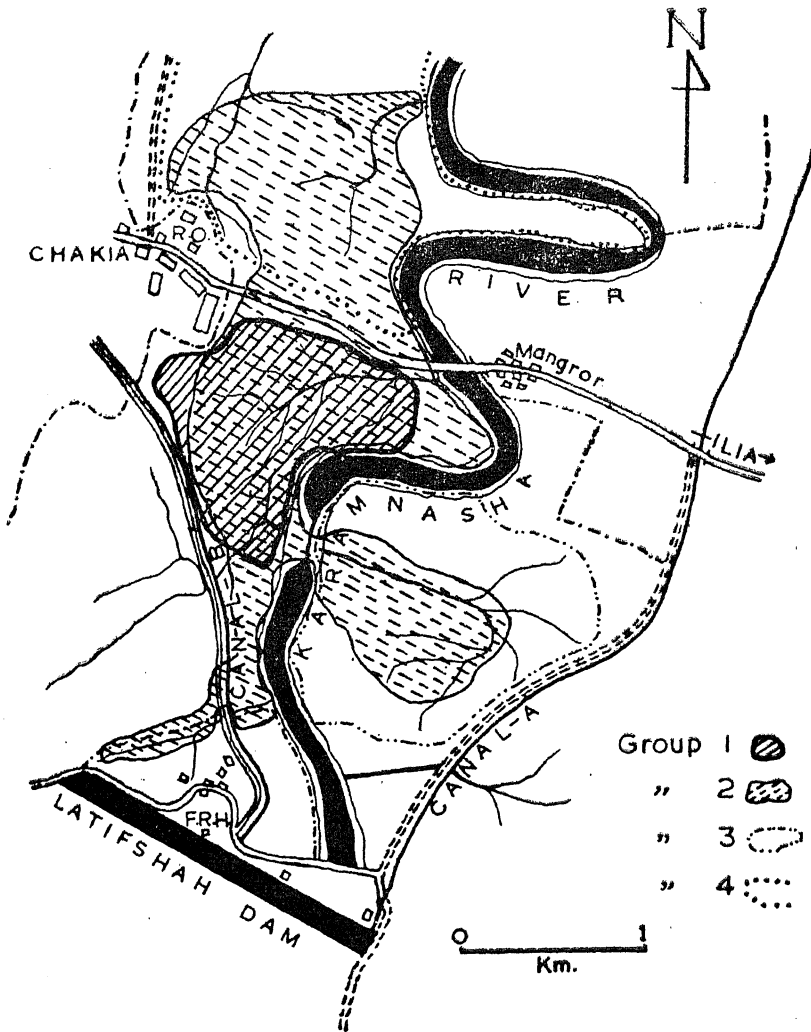


Figure 6. Home ranges of rhesus monkey groups occupying the Chakia forest.

4.4. Core area size

In Asarori forest, each group had one or two core areas, which were preferred to other parts of its home range. More than 60% of the sleeping sites converged in this area (s). The size of core areas varied from 0.04 km² to 1.81 km² with a mean of 0.48 km². There was no overlapping among core areas of different groups (figure 9). Deep ravines, high ridges, dense shrubs, tall trees of *Shorea robusta* and *Terminalia tomentosa* and presence of water were characteristic features of these core areas. The size of the core areas increased with the size of group in Asarori forest (product-moment coefficient of correlation, $r = 0.942$; $df = 11$; $p < 0.01$).

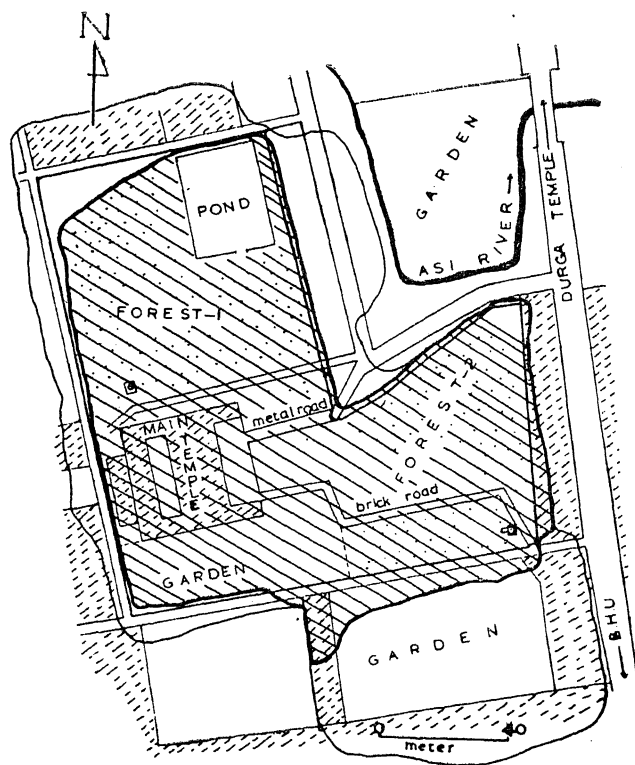


Figure 7. Home range (thin line) and core area (thick line) of the Sankat Mochan temple group.

Core areas were not discernible in the home ranges of Chakia forest monkeys. The temple monkey had permanent places to sleep during nights. The average size of the core area was 0.009 km^2 . The Sankat Mochan temple monkeys slept on the trees during nights while Durga temple monkeys slept on the temple roof.

5. Discussion

We found variability in home ranges of rhesus monkeys in different habitats. This variability can be understood partially in terms of some conclusions drawn by Clutton-Brock and Harvey (1977) for primates in general.

(i) "Populations living in areas of low food availability tend to have larger home ranges than those living in areas where food is more abundant." The Asarori forest groups lived in larger home ranges (mean 5.18 km^2) than those of temple groups (mean 0.017 km^2). Southwick and Siddiqi (1974) have also reported similar differences in the home ranges of forest and temple rhesus groups. However, conditions in Chakia forest are different due to scarce food resources, few night lodging trees and restricted space. These conditions are almost similar to those reported by Lindburg (1971) for the 4 rhesus groups living in Forest Research Institute, Dehra Dun. Although the rhesus groups at Chakia forest

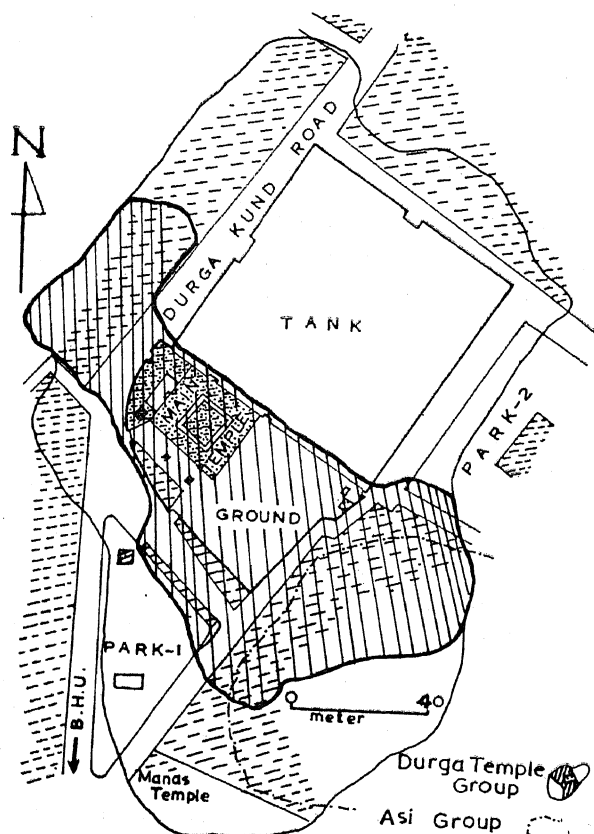


Figure 8. Home range (thin line) and core area (thick line) of the Durga temple group.

lived in home ranges, the core areas were absent. The 4 groups at Forest Research Institute lacked in both, they neither had specific home ranges nor the core areas.

(ii) "Within populations, groups whose ranges include a large proportion of preferred habitat tend to have the smallest ranges." Among the urban population of rhesus monkeys, the home ranges of temple groups were much smaller than the home ranges of groups living in other city niches. In the 2 temples plenty of food was offered to monkeys but the other 7 groups living in other parts of the Varanasi city procured their food by pilferaging from houses and shops. The latter groups were observed to roam over large areas of the city.

(iii) "Large groups may occupy bigger ranges than smaller groups though this is not always the case." We found that in Asarori forest, the size of the home range was closely correlated with the group size; the smaller the group size the smaller the home range, and *vice versa*. In a follow-up study, Makwana (1978) also observed a similar relationship between group size and home range size. Field data from other sources are not adequate to make comparisons.

(iv) "Home ranges tend to be largest at those times of the year when food is least available. When food and water were scarce in the Asarori forest during the hot dry season (March-June), the group covered the largest area of home

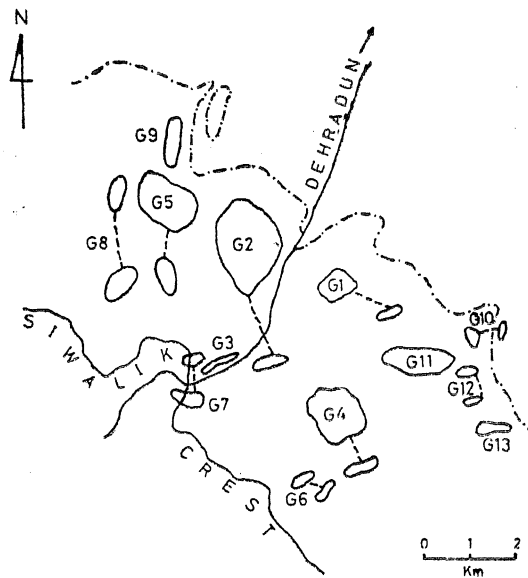


Figure 9. Core areas of rhesus monkey groups occupying the Asarori forest.

range (Lindburg 1971). Our observations also support this, however, it seems true only for those groups whose home ranges are fairly large, approximately above 5 km².

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References

- Clutton-Brock T H and Harvey P H 1977 Primate ecology and social organization ; *J. Zool. (London)* **183** 1-30
- Lindburg D G 1971 *The rhesus monkey in North India ; an ecological and behavioural study ; in Primate Behaviour : Developments in field and laboratory research II* (ed.) L A Rosenblum (New York : Academic Press) pp. 1-106.
- Lorenz K Z 1965 *Evolution and modification of behaviour* (London : Methuen)
- Makwana S C 1978 Field ecology and behaviour of rhesus macaque (*Macaca mulatta*) : 1. Group composition, home range, roosting sites and foraging routes in the Asarori Forest ; *Primates* **19** 483-492
- Pirta R S and Singh M 1978 Establishment of home range, intraspecific and interspecific relations in rhesus monkeys (*Macaca mulatta*) under infant-infant rearing conditions ; *Proc. Indian Acad. Sci.* **B87** 267-278
- Pirta R S and Singh M 1980 Changes in home ranges of rhesus monkey (*Macaca mulatta*) groups living in natural habitats ; *Proc. Indian Acad. Sci.* **B89** 515-525

- Rahman H and Parthasarthy M D 1978 Behavioural variants of bonnet macaque (*Macaca radiata*) inhabiting cultivated gardens ; *J. Bombay Nat. Hist. Soc.* **75** 406-425
- Southwick C H and Siddiqi M F 1974 Contrasts in primate social behaviour ; *Bioscience* **24** 398-406
- Vogel C 1977 *Ecology and sociology of Presbytis entellus* ; in *Use of non-human primates in biomedical research* (eds.) M R N Prasad and T C Anand Kumar (New Delhi : Indian National Science Academy) pp. 24-45.

Effects of aldrin on serum and liver constituents of freshwater catfish *Clarias batrachus* L.

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Abstract. The changes in total protein, total phosphorus, calcium and cholesterol contents were observed in serum and liver of treated and control fish *Clarias batrachus* L. Aldrin caused more pronounced effect in liver than in serum. Values remained significantly low in liver of experimental fish than in control, while the serum constituents fluctuated widely. The variations observed in different values are explained as transfer or development of tolerance. A constant increase of cholesterol in serum corresponding to a regular decrease in liver is interpreted as deportation of cholesterol from liver to serum as a result of liver damage by aldrin poisoning.

Keywords. Aldrin effects ; serum ; liver constituents ; *C. batrachus*.

1. Introduction

Investigations have proved that chlorinated hydrocarbons are highly toxic to fish. Acute toxicity causes damage to the central nervous system, resulting in instability, respiratory difficulties and sluggishness. Other chronic effects are residue accumulation in fats, damage to liver and kidneys, reduced reproduction and restricted growth (Donald 1968). Besides, many alterations have also been reported to occur in blood and tissue chemistry as a result of toxicants. A haemoglobin decrease without a change in erythrocyte count was observed due to the effect of DDT (Rudd and Genelly 1956). Exposure to endrin resulted in increased concentration of sodium, potassium, calcium, and cholesterol in serum with a lower values of sodium, potassium, calcium and zinc in the liver of northern puffer *Sphaeroides maculatus* than control (Eisler and Edmunds 1966). Changes in serum proteins and free amino acids were reported in *Channa punctatus* after exposure to malathion, endrin and dieldrin (Shakoori *et al* 1976). In the same fish Lone and Javaid (1976) observed the variation in blood caused by the effect of DDT and dieldrin.

But so far no such investigation has been reported on aldrin. We report here the changes in chemical constituents of serum and liver tissue of a catfish *Clarias batrachus* which was exposed to various concentrations of aldrin.

2. Material and methods

C. batrachus (23–25 cm in length and weighing 80–100 g) were collected from a local pond and acclimatized for two weeks in a large size aquarium. Fish meal was provided daily up to 24 hr before the aldrin administration.

15 fish were maintained in each of the 5 buckets, containing 20 litres of tap water (pH 6.7, water temperature 25–30°C). Appropriate quantity of technical grade aldrin was dissolved in acetone and the final concentrations of 0.1, 0.2, 0.5 and 1 ppm were added to each of four buckets. The control fish (5th bucket) received only 1 ml of acetone. No fish meal was provided during the experiment. 5 fish from each treatment were sacrificed after 12, 60 and 132 hr of aldrin exposure.

Blood from each fish was collected in a clean test tube after severing the caudal peduncle and was placed in a refrigerator at 10°C for 24 hr. After centrifugation at 3500 r.p.m. for 15 min, serum was drained out and returned to the refrigerator for storage at 4°C. The liver was removed, washed with physiological saline solution and kept in refrigerator till use. The total cholesterol in serum and liver was estimated using the method of Zaltikis *et al* (1953). Other chemical constituents were measured using methods as described by Oser (1965).

3. Results

The 24 hr lethal concentration (LC_{100}) for *C. batrachus* was observed to be 1 ppm aldrin. At lower concentrations, no mortality occurred in experimental fish except for two additional counts at 0.5 ppm.

3.1. Observations on serum

As indicated in figure 1, the serum constituents varied markedly with doses and exposure time. At 12 hr exposure the serum protein showed a gradual fall ($P < 0.01$) in all concentrations. Longer exposure (60 and 132 hr) exhibited a rapid fall ($P > 0.01$) in total protein for 0.1 ppm, the constituent rose higher for 0.2 ppm and declined again for 0.5 ppm ($P > 0.01$).

The value of total phosphorus was found increasing with increasing concentration at different exposure times. Total phosphorus, which showed a continuous increase with concentration of 12 hr exposure, declines slightly for 60 hr exposure at 0.2 ppm and more markedly at 0.5 ppm. At 132 hr exposure a small but constant decline of trend was observed for concentration higher than 0.1 ppm.

The cholesterol level showed a continuous increase with aldrin concentration and exposure time, being maximum in fish exposed for 132 hr and minimum in 12 hr exposed fish ($P > 0.01$).

Calcium content declined steadily in fish treated with 0.1 ppm after 12 hr and increased later with rising concentration. At 60 hr exposure the level rose for 0.1 and 0.2 ppm and thereafter declined a little, remaining all the time higher than the control value as well as the corresponding values for 12 hr exposure. For 132 hr exposure the slight increase observed at 0.1 ppm (higher than 60 hr values) was followed by a continuous declining trend for higher concentrations.

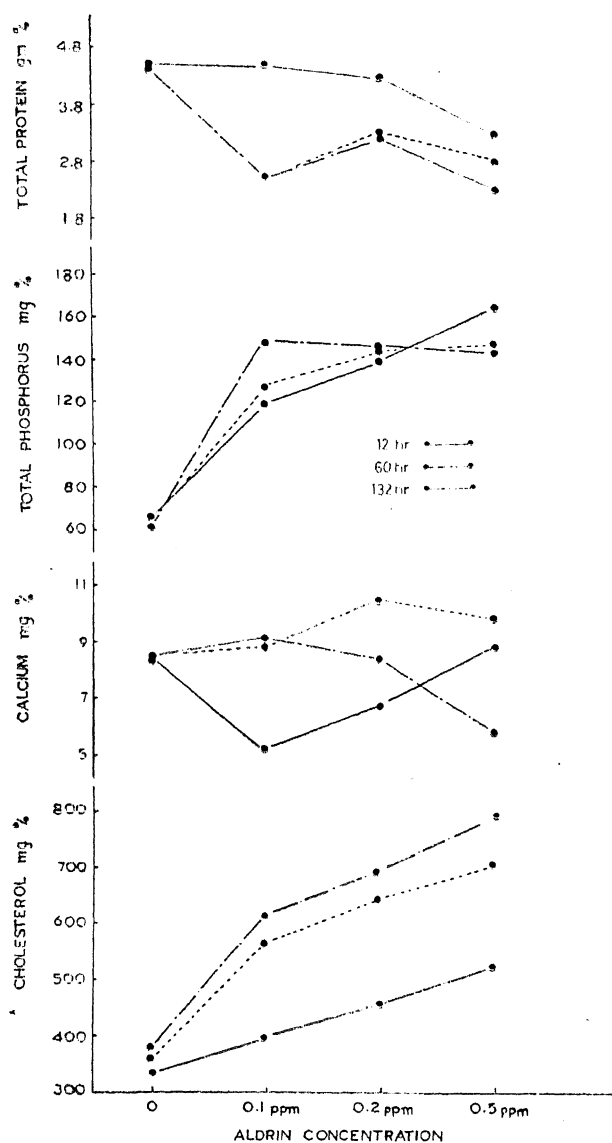


Figure 1. Serum constituents of *Clarius batrachus* for different exposure time.

3.2. Observations on liver

The liver constituents exhibited a more regular pattern of variation (figure 2). In all experimental fish the values of protein, calcium and cholesterol content decreased gradually with increasing aldrin toxicity at different exposure times. The value of total protein and calcium was significantly higher for 60 hr exposure ($P > 0.05$) than 12 and 132 hr. The decline of protein content for 12 hr exposure was steep and steady up to 0.2 ppm. Thereafter values remained low at higher

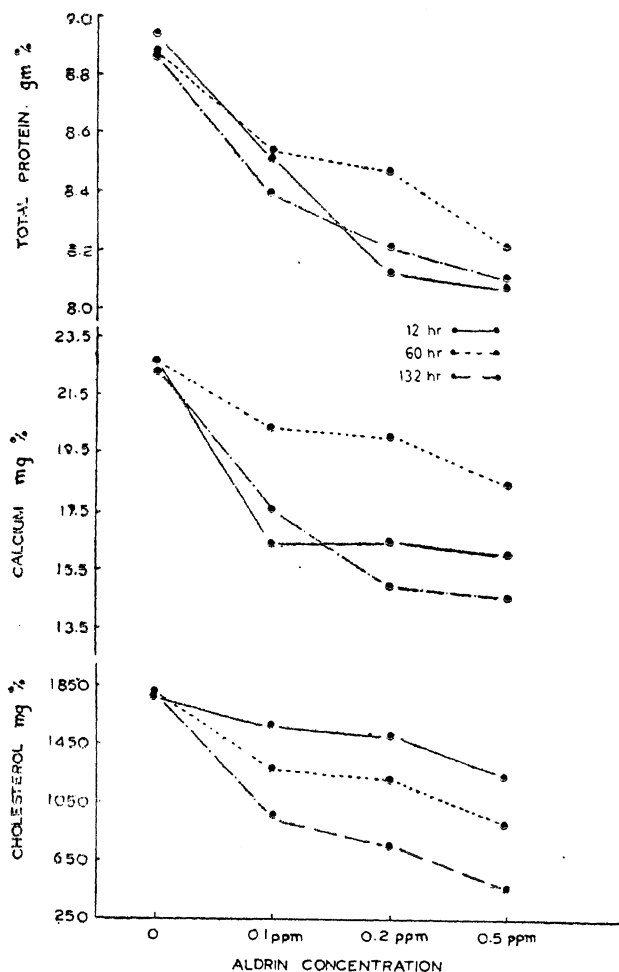


Figure 2. Liver constituents of *Clarias batrachus* for different exposure time.

concentrations. Whereas at this exposure calcium content decreased only up to 0.1 ppm and after that the values were found uniform with rising concentrations.

A slow and steady decrease in cholesterol level was observed both with exposure time and concentration, values for 12 hr exposure being highest and those for 132 hr the lowest ($P > 0.001$).

4. Discussion

Boyle *et al* (1966) reported that in a fish tissue aldrin starts converting into dialdrin 8 hr after the fish is exposed to it, and the conversion reaches 94% in about 32 days. In our observation also, this conversion must have taken place a little in 12 hr and considerably more for 60 and 132 hr in increasing proportion.

The median tolerance limit of four species of fish has been reported 24 hr at aldrin concentrations ranging from 0.089 to 0.018 ppm and for dialdrin 0.062

to 0.014 ppm (Gakstatter 1968). The present paper describes time dependence of tolerance towards aldrin and dieldrin as shown by liver and serum and other tissues by inference.

The level of protein decreased 25 to 50% in serum and 8 to 10% in liver correspondingly (figures 1 and 2). Low levels in serum protein have also been reported in goldfish (Grant and Mehrle 1970). The protein graphs for serum and liver show quite a different pattern of variation with concentration and time. In serum for a 12 hr exposure, the effect of aldrin is rather small at 0.1 and 0.2 ppm and becomes more marked at 0.5 ppm in a systematic and expected manner. The effect is found to be markedly enhanced at longer exposure thereby showing an important time-dependence of the aldrin affect. At 0.1 ppm, the protein values are the same after 60 and 132 hr. From this it is inferred that at this dose aldrin and its by-product cease to effect protein after 60 hr exposure. The upward bend of graphs for higher concentrations indicates fish tolerance to the chemical which is time dependent as well as concentration dependent. For a longer exposure time and higher concentration, the tolerance starts declining.

In liver the protein graph showed no evidence of tolerance developed at 0.1 and 0.2 ppm doses for 12 hr exposure, therefore steep constant decline. At a higher dose, sufficient amount of antibodies appear to be formed to check a further decline of protein to an appreciable extent. For 60 hr exposure, the time is long enough to permit the interference by produced antibodies even at 0.1 ppm level. This raises the observed protein values at this concentration as compared to 12 hr. This tendency continues up to 0.2 ppm indicating a maximum tolerance near this point after which the tolerance declines resulting in low protein values. For 132 hr exposure the tolerance cycle appears to have come down to zero again at 0.1 ppm drug level to permit a little more decline of protein value than for 12 hr. At 0.2 and 0.5 ppm, the tolerance appears to have decreased appreciably below its maximum value, but it remains effective enough to place the 0.2 and 0.5 ppm points for 132 hr above those for 12 hr.

The higher level of total phosphorus in the serum of aldrin-treated fish appears to be related to the changes in liver produced under the effect of toxicant. Induction of serum aminotransferases (SGOT and SGPT) lactic dehydrogenase and alkaline phosphatase are reported to be as a result of hepatic changes caused by pesticides (Matsumura 1975).

The continuous increase in total phosphorus for 12 hr exposure means a large concentration is almost proportionately more effective in causing the damage that is releasing phosphorus. Regarding the time factor it is noticed that at 0.1 ppm the released phosphorus increases with period of exposure though not in proportion to the time but at a reduced rate. At 0.2 ppm, an increase in the released quantity of phosphorus is observed with time, but slightly less in quantity from 12 to 60 hr exposure, and very little in going from 60 to 132 hr. At 0.5 ppm the trend is reverse and the released phosphorus decreases from 12 to 60 and then from 60 to 132 hr.

Figures 1 and 2 reveal that for a 12 hr exposure the calcium value declines to about 36% in serum and 29% in liver at 0.1 ppm dose. The level remains constant in liver for higher concentrations up to 0.5 ppm but rises steadily in serum correspondingly. This precludes a direct transfer from liver to blood or *vice versa* for this exposure due to the abrupt liver damage. The constant level

in liver can be interpreted as the stoppage to further liver damage due to the production of antibodies under higher doses called tolerance. The rise of calcium content in serum may be at least partly also due to a similar reason but a sizeable part of the excess calcium should result from various tissues, other than liver because no loss of calcium at the corresponding point is seen (figure 2). For 60 hr exposure the values of liver calcium are related to about maximum tolerance and 132 hr values associated to tolerance that comes down again to almost zero.

In serum the calcium values were observed to be higher than the control value at certain concentrations and exposure times. This peculiarity is hard to explain on tolerance basis and the transfer of calcium appears evident in these conditions from other tissues to serum. The decline for longer period will then indicate a greater excretion.

On comparing figure 1 with figure 2 it is seen that the trends of cholesterol variation in liver and serum are opposite to each other. Almost a regular increase observed in serum at different concentrations and exposures, contrasts to a regular decrease in liver. This is a clear case of transfer of cholesterol from liver to serum and from other organs and confirms the observations of Eisler and Edmunds (1966) on puffers with endrin.

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References

- Boyle H W, Burttschell R and Rosen A A 1966 Infrared identification of chlorinated insecticides in tissues of poisoned fish. In organic pesticides in the environment; *Advan. Chem. Ser.* 60 American Chemical Society Chap. 17
- Donald W J 1968 Pesticides and fishes—A review of selected literature; *Trans. Amer. Fish. Soc.* 97 398–424
- Eisler R and Edmunds H 1966 Effect of endrin on blood and tissue chemistry of marine fish; *Trans. Amer. Fish. Soc.* 95 153–159
- Gakstatter J H 1968 Rates of accumulation of 14 C—dieldrin residues in tissues of gold-fish exposed to a single sublethal dose of 14C—aldrin; *J. Fish. Res. Board. Can.* 25 1797–1802
- Grant B F and Mehrle P M 1970 Chronic endrin poisoning in gold-fish *Carassius auratus*; *J. Fish Res. Board Can.* 27 2225
- Lone K P and Javaid M Y 1976 Effect of sublethal doses of DDT and dieldrin on the blood of *Channa punctatus* (Bloch); *Pak. J. Zool.* 8 143–9
- Matsumura F 1975 *Toxicology of insecticides* (New York and London; Plenum Press)
- Oser B L 1965 *Hawks physiological chemistry* 14th ed. (New York: McGraw-Hill)
- Rudd R L and Genelly 1956 Pesticides their use and toxicity in relation to wildlife; *Calif. Fish. Gama. Bull.* 7 1–309
- Shakoori A R, Zaheer S A and Ahmad M S 1976 Effect of malathion dieldrin and endrin on blood serum proteins and free amino acids pool of *Channa punctatus* (Bloch); *Pak. J. Zool.* 8 125–34
- Zaltikis A, Zak B and Bayl A J 1953 A new method for the direct determination of serum cholesterol; *J. Lab. Clin. Med.* 41 486

Hepatopancreatic sucrase of *Macrobrachium lamarrei* (Crustacea, Caridea, Palaemonidae)

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Abstract. The effect of seven factors was studied on the activity of hepatopancreatic sucrase of *Macrobrachium lamarrei*. Its optimum pH is 6.0 and optimum temperature 50°C and its activity was suppressed by the two end products, glucose and fructose. On prolonging incubation period sucrase activity remained constant up to 8 hr, declined thereafter, finally becoming zero. Increasing enzyme concentration produced a similar effect. Its K_m value is 4.5×10^{-2} M. Dialysis suppressed its activity by 17.9%.

Keywords. Crustacea ; caridea ; palaemonidae ; *Macrobrachium lamarrei* ; sucrase ; hepatopancreatic sucrase.

1. Introduction

The enzyme sucrase (β -fructofuranosidase, EC 3.2.1.26) is known to occur in the digestive system of a wide variety of crustaceans (Mansour-Bek 1954; van Weel 1970; Vonk 1960). Yet information on its kinetic properties is scanty, being confined to its response to pH (Agarwal 1963, 1964; Martin 1966; Newcomer 1956; Nicholls 1931; Wiersma and van Ween 1928). The present work was conducted to determine the properties of this enzyme by studying the effect of seven factors on hepatopancreatic sucrase of *Macrobrachium lamarrei*, a freshwater shrimp, reported to be rich in this enzyme (Murthy 1978).

2. Materials and methods

Hepatopancreatic glands from 100 *Macrobrachium lamarrei* Milne Edward were pooled in ice cold distilled water, dried between filter-paper sheets, weighed and homogenized in distilled water. The homogenate was centrifuged at 3000 $\times g$ for 15 min at 4°C and the supernatant diluted to a concentration of 10 mg (wet weight)/ml (or 0.1 ml \equiv 1 mg) of hepatopancreas. The assay system consisted of: appropriate buffer 0.3 ml, 0.3 M sucrose 0.2 ml and enzyme extract 0.1 ml; in controls a heat denatured enzyme was added. After incubating the mixtures at 37°C for 4 hr, following Bernfield's (1955) colorimetric method for estimating hexose sugars, the reaction was stopped by adding 0.5 ml of 3,5-dinitrosalicylic reagent; thereafter the mixtures were warmed for 15 min, the volume raised to

6 ml with distilled water and readings taken at 540 nm. Under conditions similar to those for the enzyme assay, direct reaction of glucose with dinitrosalicylic reagent gives 1 extinction unit = 0.6 mg glucose. Of the seven factors—pH, temperature, end products, incubation period, enzyme and substrate concentration and dialysis—pH was the first factor to be studied, in order to ascertain its optimum value, at which the effect of the six remaining factors was investigated. Some experimental details are given in § 3. The presented values of each factor represent the mean of five replicates.

3. Observations

3.1. Determination of optimum pH

According to the results using two buffer series (0.1 M sodium citrate buffer from pH 3.5–6.5 and 0.1 M Sorenson's phosphate buffer from pH 5.5–8.0), sucrase remained quite active from pH 4.5–7.0 and its optimum activity occurred at pH 6.0 (figure 1). While its optimum pH with both buffers coincided, enzymic activity at this pH (6.0) with phosphate buffer was 9.7% greater than that using citrate buffer.

3.2. Effect of temperature

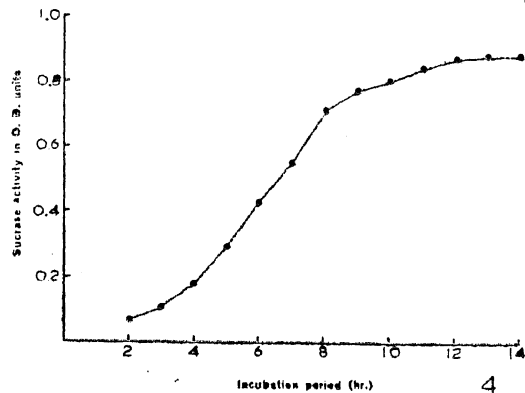
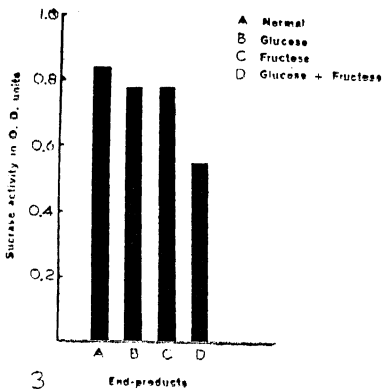
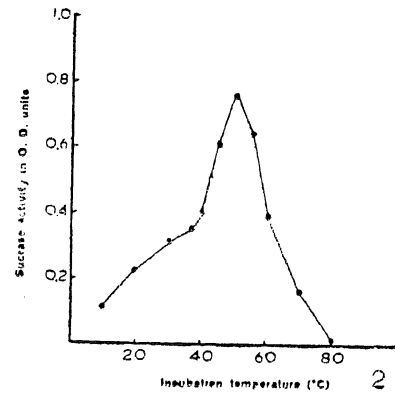
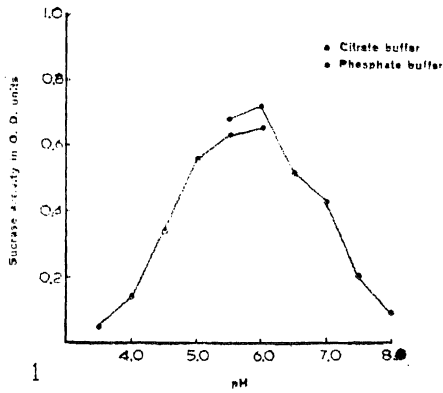
Enzymic activity was tested at eleven temperatures ranging from 10°–80° C (figure 2). Sucrase showed optimum activity at 50° C, although remaining quite active from 20°–6° C. The activity increased slowly from 10°–40° C but steeply from 40°–50° C; above 50° C it fell sharply becoming almost zero at 80° C.

3.3. Effect of end products

Four tubes, *A* to *D*, of the assay system were prepared and to three, *B* to *D*, the end product(s) were added before their incubation. No end product was added to tube *A*, being meant to serve as the blank for calculating normal enzymic activity. In the parallel controls of *B* to *D*, the end product(s) were added after their incubation. The details are tabulated below :

	Tube <i>A</i>	Tube <i>B</i>	Tube <i>C</i>	Tube <i>D</i>
1% Glucose, in ml	0.0	0.1	0.0	0.05
1% Fructose, in ml	0.0	0.0	0.1	0.05
Distilled water, in ml	0.2	0.1	0.1	0.1

The solutions of glucose and fructose when added separately caused inhibition of sucrase activity to the same extent, by 7.2%; whereas a mixture of both caused 34% inhibition (figure 3).



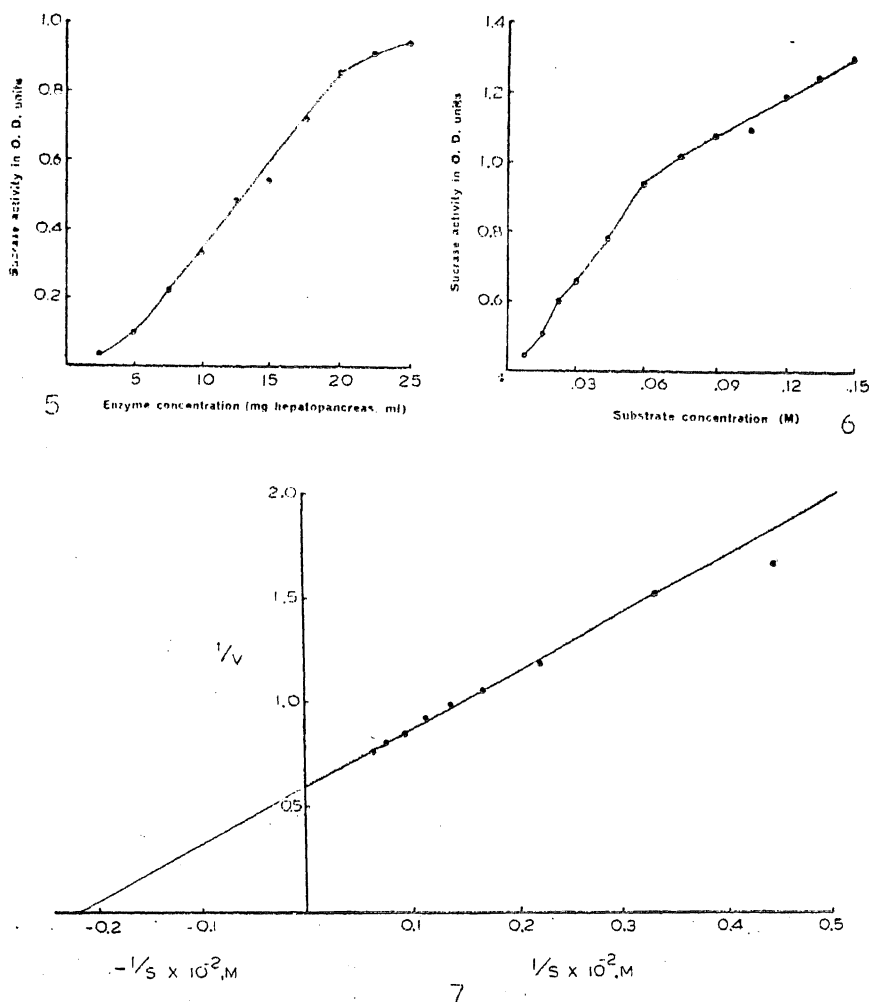
Figures 1-4. 1. Effect of pH on hepatopancreatic sucrase. 2. Effect of temperature on hepatopancreatic sucrase. 3. Effect of end products on hepatopancreatic sucrase. 4. Effect of incubation period on hepatopancreatic sucrase.

3.4. Effect of incubation period

Results of incubating 13 tubes for progressively longer duration by one hour show that from 2-8 hr the rate of increase of formation of end products was more or less linear (figure 4). Thereafter, the build-up of hexose sugars decreased becoming constant after 14 hr of incubation. This pattern indicates a somewhat constant rate of enzymic activity up to 8 hr, followed by decreased activity, reaching finally close to zero at 14 hr.

3.5. Effect of enzyme concentration

Enzyme extracts of ten concentrations, ranging from 2.5 mg/ml to 25 mg/ml of hepatopancreas, were tested (figure 5). The liberation of hexose sugars increased more or less linearly up to an enzyme concentration of 20 mg/ml. Above it the increase was considerably slower, becoming almost nil at 25 mg/ml.



Figures 5-7. 5. Effect of sucrase concentration on its activity. 6. Effect of substrate concentration on hepatopancreatic sucrase. 7. Lineweaver-Burk plot for Michaelis constant (K_m value) of hepatopancreatic sucrase.

3.6. Effect of substrate concentration

Sucrose solutions of 12 concentrations, ranging from 0.0075 to 0.15 M, were tested. The rate of liberation of hexose sugars was almost linear up to 0.06 M concentration; after which it gradually slowed down (figure 6). The Michaelis constant (K_m value) of hepatopancreatic sucrase as calculated from the collected data is 4.5×10^{-2} M (figure 7).

3.7. Effect of dialysis

An enzyme preparation dialysed for 24 hr at 4°C against double distilled water suffered a 17.9% loss in activity.

4. Discussion

In *Astacus fluviatilis* the optimum activity of gastric juice sucrose was reported to occur at pH 6.0 (Wiersma and van Ween 1928), as well as over a small pH range 4.5-5.0 (Krüger and Graetz 1928). The optimum activity of hepatopancreatic sucrose of *Marinogammarus obtusatus* (4.2-6.4; Martin 1966), *Porcellio laevis* (5.5-6.5; Newcomer 1956) and of *Thalamita crenata* (7.74-7.87; van Wael 1960) takes place over a narrow pH range. In contrast, that of *Corophium volutator* and *Orchestia gammarella* (5.8, 6.0 respectively; Agarwal 1963, 1964), *Ligia oceanica* (5.8; Nicholls, 1931) occurs at a sharp pH. The optimum pH of hepatopancreatic sucrose of *M. lamarrei* being 6.0 is a sharp peak. Being lower than the pH of the stomach contents (6.4-6.7; Murthy 1978), sucrose activity *in vivo* would therefore be about 66.6-72.25% of its full activity *in vitro*.

The effect of the six remaining factors apparently remains uninvestigated on crustacean sucrose. However, as the effect of three of them, temperature, end products and dialysis has been studied on insectan sucrose, the findings on *M. lamarrei* have been compared with the available insectan data. The optimum temperature of hepatopancreatic sucrose of *M. lamarrei* corresponds to that of *Sarcophaga ruficornis* and *Musca domestica* (50° C; Sinha 1976), but is higher than that of *Blatella germanica* (25° C; Day and Powning 1949), *Sesamia inferens* (30° C for gut, 35° C for salivary glands; Agarwal 1976), *Acyrtosiphon pisum* (35° C; Srivastava and Auclair 1962), *Chrysomphalus aonidum* and *Aonidiella aurantii* (37° C; Ishaaya and Swirski 1970) and *Lygus disponi* (37° C; Hori 1971).

Inhibition by the end products of sucrose activity, as occurs in *M. lamarrei*, has been recorded in insects like *Bombyx mori* (Horie 1959), *Dysdercus fasciatus* (Khan and Ford 1967), *A. pisum* (Srivastava and Auclair 1962) and *S. inferens* (Agarwal 1976); no effect was recorded in *Aedes aegypti* (Yang and Davies 1968) and *L. disponi* (Hori 1971) by their accumulation.

The results in the case of three factors, (i) prolonged incubation, (ii) increasing concentration of enzyme and (iii) substrate are similar, as an initial fast hydrolysis of the substrate undergoes slowing down. The slowing down after prolonged incubation can be due either to the inhibitory effect of glucose and fructose formed or to a depletion of sucrose or to a combination of both. The retardation by relatively higher concentrations of the substrate can be due to the conversion of the total enzyme into ES-complex, as postulated by Karlson (1969). However, accumulation of formed hexose sugars can be a contributory factor. That by stronger enzyme extracts can be attributed either to rapid exhaustion of the substrate due to excessive enzyme or to the inhibitory effect of the formed end products or to a combination of both.

At present, reduced activity of hepatopancreatic sucrose of *M. lamarrei* after dialysis can neither be explained nor compared. However, dialysed sucrose from the gut and salivary glands of lepidopterous larvae showed 8% activation and 37.5% inhibition respectively after dialysis (Agarwal 1976).

References

- Agarwal A K 1976 Effect of various factors on the activity of sucrase from the larvae of *Sesamia inferens*; *Ent. Exp. Appl.* 20 19-30
- Agarwal V P 1963 Determination of optimum pH for the activity of caecal invertase of *Corophium volutator* Pallas; *J. Anim. Morphol. Physiol.* 10 86-88
- Agarwal V P 1964 Determination of optimum pH for the activity of caecal carbohydrases of the amphipod *Orchestia gammarella*; *J. Zool.* 143 545-551
- Bernfield P 1955 Amylases α - and β - *Methods in enzymology* (eds.) S R Colowick and N O Kaplan (New York: Academic Press) 1 149-158
- Day M F and Powning R F 1949 A study of process of digestion in certain insects; *Aust. J. Sci. Res.* B2 175-215
- Hori K 1971 Nature of gut invertase of *Lygus disponi* (Hemiptera: Miridae); *Res. Bull. of Obihiro Zootech. Univ. Ser.* 1 666-675
- Horie Y 1959 Physiological studies on the alimentary canal of the silk worm *Bombyx mori*. II. Carbohydrases in the digestive fluid and in the midgut tissue; *Bull. Sericult. Exp. Sta. Jpn.* 15 365-381
- Ishaaya I and Swirski E 1970 Invertase and amylase activity in the armour scales *Chrysomphalus aonidum* and *Aonidiella aurantii*; *J. Insect Physiol.* 16 1599-1606
- Karlson P 1969 *Introduction to modern Biochemistry* 3rd edition (New York: Academic Press)
- Khan M A and Ford J B 1967 The distribution and localisation of digestive enzymes in the alimentary canal and salivary glands of the cotton stainer, *Dysdercus fasciatus*; *J. Insect Physiol.* 13 1610-1628
- Krüger P and Graetz E 1928 Die Fermente des Flusskrebsmagensaftes; *Zool. Jahrb. Abt. Allg. Zool. Physiol. Tiere* 45 463-514
- Mansour-Bek J J 1954 Digestion in Invertebrates; *Tabulae Biologicae* 21 173-195
- Martin A L 1966 Feeding and digestion in two intertidal gammarids *Marinogammarus obtusatus* and *M. pirloui*; *J. Zool.* 148 515-525
- Murthy R C 1978 Study of carbohydrases in the digestive system of *Macrobrachium lamarrei* Edwards (Crustacea: Decapoda); *Comp. Physiol. Ecol.* 3 13-16
- Newcomer W S 1956 Digestive carbohydrases of the wood louse, *Porcellio laevis* (Crustacea: Isopoda); *Physiol. Zool.* 29 157-162
- Nicholls A G 1931 Studies on *Ligia oceanica*. Part II. Process of feeding, digestion and absorption with a description of the foregut; *J. Mar. Biol. Ass. U.K.* 17 675-707
- Sinha M 1976 Invertase activity in the midgut of *Sarcophaga ruficornis* and *Musca domestica* (Diptera: Insecta); *Experientia* 32 341-342
- Srivastava P N and Auclair J J 1962 Characteristics of invertase from the alimentary canal of the pea aphid, *Acyrtosiphon pisum* (Harr.) (Homoptera: Aphididae); *J. Insect Physiol.* 8 527-536
- van Weel P B 1960 On the secretion of digestive enzymes by the marine crab *Thalamita crenata*; *Z. Vgl. Physiol.* 43 467-536
- van Weel P B 1970 Digestion in Crustacea. In *Chemical Zoology* (eds.) M Florkin and B T Scheer 5 (A), 97-113 (New York: Academic Press)
- Vonk H J 1960 Digestion and Metabolism. In *Physiology of Crustacea* (ed.) T. H. Waterman (New York: Academic Press) 1 291-311
- Wiersma C A G and van der Ween R 1928 Die Kohlenhydratverdauung bei *Astacus fluviatilis*; *Z. Vgl. Physiol.* 7 269-278
- Yang Y J and Davies D M 1968 Occurrence and nature of invertase activity in adult blackflies (Simuliidae); *J. Insect Physiol.* 14 1221-1232

Shell selection in the estuarine hermit crab *Clibanarius longitarsus* (De Haan)

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Abstract. In the laboratory, under choice situation, *C. longitarsus* preferred shells with greater shell width and shell weight. The correlation coefficient values calculated between the crab and shell parameters in the laboratory sample showed that this hermit crab selects a shell of suitable dimension for its occupation. In the multiple regression equations calculated with carapace length against the three shell variables aperture width, shell width and shell weight which are deemed to be important for hermit crabs, the regression coefficient of the variables varied much. The unscaled first principal component explained 84.9% of the total variability of the shell parameters and scaled first principal component explained 95.8% of shell parameters. The scaled first principal component was found to be a reliable estimator of shell size and the hermit crab, if given a choice, selected a shell of suitable dimension which fits its body quite closely.

Keywords. *Clibanarius longitarsus* ; shell selection in laboratory ; multivariate analysis.

1. Introduction

Empty gastropod shells constitute an important resource for hermit crabs and a hermit crab in order to protect itself from the environment and from its predators must enter a gastropod shell. This behaviour of hermit crabs living in empty gastropod shells intrigued naturalists down through the ages from Aristotle (Reese 1963) to the present (Ajmal Khan *et al* 1980). Hermit crabs do not enter gastropod shells at random but prefer shells according to shape, shell covering, dimension and weight (Grant and Ulmer 1974). Under choice situation, the shell factors influencing the estuarine hermit crab *Clibanarius longitarsus* in shell selection were studied presently through multivariate analysis.

2. Materials and methods

Five measures of the gastropod shells, i.e., shell length, shell width, aperture length, aperture width and shell weight and three measures of hermit crabs, i.e., carapace length, dactylus length of second left walking leg and weight of the crab were used

in the present study. The length of the shell was measured from the tip of spire to the other end of the shell, the shell width was the greatest distance at right angles to the length of the shell, aperture length was measured parallel to shell length and the aperture width was the greatest distance from the margin of the outer lip of the inner wall of the inner lip at 90° to aperture length (parietal callosities were excluded from aperture width). The shells were completely dried before weighing. Carapace length of the crab was measured from the tip of the rostrum to the posterior notch and the live body weight was taken after blotting the specimen dry with a blotting paper. Correlation coefficient values between crab and shell variables were calculated for 50 specimens collected from the field and for 50 specimens of hermit crabs allowed to select shells of their choice in the laboratory. For shell selection experiments in the laboratory, the crabs were divided into 4 size groups (I group 4-6.9 mm, II group 7-10 mm, III group 10.1-14.9 mm and IV group 15 mm and above) and the gastropod shells inhabited in the field by crabs in each length were identified and the empty gastropod shells represented in each length group were collected and used in the shell selection experiments. Percentage distribution of molluscan shell types, occupied by the crabs of the four size groups is given elsewhere (Ajmal Khan and Natarajan 1981). Shell selection experiments in the laboratory were done following Grant and Ulmer (1974).

3. Results

Shell preference of the hermit crab was found to vary from one size group to another. While the first size group crabs showed preference for the shells of *Nassa jacksoniana*, second, third and fourth size groups showed preference for the shells of *Nassa dorsata*, *Babylonia spirata* and *Bursa spinosa* respectively.

The mean values of carapace lengths of crabs and variables of gastropod shells occupied by the hermit crabs in the field and selected in the laboratory experiments, are given in table 1. Since the standard errors of the shells selected in the laboratory were noticeably larger than the corresponding values in field sample, a modified form of the *t* test, the statistic *d* (Bailey 1959) was used to determine if the carapace length and shell parameters of the two categories were significantly different from each other. It was found that the carapace length of hermit crabs in the above two situations were not significantly different. In the same way among the shell variables, the aperture width in these two categories was not significantly different but the shell width and shell weight differed significantly.

The correlation coefficient values calculated between the crabs and shell parameters in field samples and laboratory selected samples are given in table 2. It was found that there was no large scale difference in the *r* values of aperture width and shell width calculated against carapace length in the two situations and in both the cases the values were highly significant ($P < .001$). Eventhough the *r* values in the first category (field sample) were statistically significant in all cases except one (carapace length/shell length), the *r* values in the second category were highly significant. By looking at the *r* values in the second situation, it could be inferred that a single factor alone was not influencing this hermit crab in shell selection and this hermit crab normally selected a shell suitable in general dimension. But the difficulty in putting forward the above inference only by looking

Table 1. Results of crab carapace lengths and shell variables of shells occupied and shell selected by *C. longitarsus*. Table values are means \pm standard errors. The "d" values are those given from the formula of Bailey (1959) for comparing two means. P: Probability.

Variable	Shells occupied	Shells selected	d value	P
Carapace length	13.13 mm ± 0.91	13.12 mm ± 0.99	0.01	> 0.10
Shell width	20.98 mm ± 0.66	28.26 mm ± 2.39	2.94	< 0.01
Aperture width	9.68 mm ± 0.29	12.04 mm ± 1.10	1.90	> 0.05
Shell weight	8.88 mm ± 0.78	18.31 mm ± 3.12	2.93	< 0.01

Table 2. Correlation coefficient values between the crab and shell parameters of shells occupied and shells selected by *C. longitarsus*. P: Probability; Number of sample 50 each.

Parameters	r value in field sample	P	r value calculated after shell selection experiment in the laboratory.	p
Carapace length/Aperture length	+0.413	< 0.01	+0.960	< 0.001
Carapace length/Aperture width	+0.636	< 0.001	+0.890	< 0.001
Carapace length/Shell length	+1.356 (Spurious)	..	+0.922	< 0.001
Carapace length/Shell width	+0.464	< 0.001	+0.962	< 0.001
Carapace length/Shell weight	+0.397	< 0.01	+0.819	< 0.001
Dactylus length/Aperture length	+0.301	< 0.05	0.924	< 0.001
Dactylus length/Aperture width	+0.331	< 0.02	+0.902	< 0.001
Dactylus length/Shell length	+0.318	< 0.05	+0.904	< 0.001
Dactylus length/Shell width	+0.326	< 0.02	+0.962	< 0.001
Dactylus length/Shell weight	+0.328	< 0.02	+0.837	< 0.001
Crab body weight/Shell weight	+0.352	< 0.02	+0.815	< 0.001

at the r values was that shell variables are interrelated with each other and simple regression and correlation techniques fail to show the individual influence of a shell parameter during selection. So, for this type of study multivariate analysis seems to be preferable.

Table 3. Results of multiple regression of carapace length (Y) against aperture width (x_1), shell width (x_2) and shell weight (x_3) of gastropod shells occupied in the field and selected in the laboratory by *C. longitarsus*.

	Regression equation	Coefficient of variation of shell parameters
Shells occupied in the field	$Y - \bar{Y} = 0.012 (x_1 - \bar{x}_1)$ $+ 0.343 (x_2 - \bar{x}_2)$ $+ 0.023 (x_3 - \bar{x}_3)$	$x_1 = 63.07$ $x_2 = 60.08$ $x_3 = 56.78$
Shells selected in the laboratory	$Y - \bar{Y} = 0.395 (x_1 - \bar{x}_1)$ $+ 0.351 (x_2 - \bar{x}_2)$ $+ (-0.073) (x_3 - \bar{x}_3)$	$x_1 = 64.87$ $x_2 = 59.76$ $x_3 = 82.96$

Through studies on the shell selection behaviour of hermit crabs, it became clear that, among different shell variables, three variables, viz., shell volume, shell aperture width and shell weight were more important. The volume of shell interior is certainly of prime importance to hermit crabs, but volume estimate is difficult time consuming and often inaccurate and imprecise (Kuris and Brody 1976). So a change of shell variable was made and instead of shell volume, shell width in addition to the other two variables was used in the multivariate analysis of the present study.

The multiple regression equation of carapace length against the three shell variables in the field situation and by choice in the laboratory are given in table 3. The coefficient of variation in both the equations for aperture and shell widths did not differ widely, but the coefficient of variation for shell weight differed widely. Moreover, in the laboratory choice situation, even though the r values used in the multiple regression equation did not vary much, regression coefficient for weight differed widely from the other two. The main problem in using multiple regression to distinguish the effects of separate parameters was that there was high correlation between the three shell variables which obscured their separate effects. To overcome this difficulty principal component analysis was used.

For principal component analysis all the shell variables have to be expressed in the same units. Here the shell width and aperture width were measured in millimeters and shell weight in grams. To overcome this defect caused by the three shell variables measured in different units, scaling of shell variables has to be done and both the unscaled and scaled principal component analyses have to be compared. Scaling can be done by dividing the shell parameters by the square root of the sums of squares of their deviation (Mitchell 1976). Logarithmic transformation of the variables also approximately satisfies these conditions (Kuris and Brody 1976). In the present study scaling was done by the second method. The results of the unscaled and scaled principal component analyses are given in tables 4 and 5 respectively. Multiple regression equation of the carapace length of the crabs against the unscaled and scaled principal components are given in table 6.

The first principal component in the unscaled situation explained 84.9% of the total variability of the shell parameters. The second component explained only

Table 4. Principal components of shells selected by *C. longitarsus* in the laboratory. No Scaling has been performed on them.

Principal components		Percentage contributions to total variability of shell parameters
I Component	$1.000000x_1$	84.9
	$+0.964919x_2$	
	$+0.887572x_3$	
II Component	$-0.139600x_1$	1.5
	$+0.666600x_2$	
	$+1.000000x_3$	

Table 5. Principal components of shells selected by *C. longitarsus* in the laboratory. Scaling was performed by logarithmic transformation of shell and crabs variables. x_1 —aperture width ; x_2 —shell width and x_3 —shell weight.

Principal components		Percentage contributions to total variability of shell parameters
I Component	$1.000000 x_1$	95.800
	$+0.999043 x_2$	
	$+0.992294 x_3$	
II Component	$1.000000 x_1$	0.013
	$+ -0.500000 x_2$	
	$+0.500000 x_3$	

Table 6. Regression equations of carapace length against unscaled and scaled principal components for shells selected in the laboratory by *C. longitarsus*. Y —carapace length ; x_1 —I principal component ; x_2 —II principal component.

Regression equation		Coefficient of variation of principal components
Unscaled	$Y - \bar{Y} = 0.054 (x_1 - \bar{x}_1)$	$x_1 = 77.11$
	$+7.550 (x_2 - \bar{x}_2)$	$x_2 = 74.01$
Scaled	$Y - \bar{Y} = 0.154 (x_1 - \bar{x}_1)$	$x_1 = 61.39$
	$+0.005 (x_2 - \bar{x}_2)$	$x_2 = \text{negligible}$

1.5% of the total variability. In scaled component analysis, the results were still more significant; the first principal component explained 95.8% of the total variability while the percentage of variability of the shell parameters explained by the second component was very low (0.013%). Moreover both in unscaled and scaled first principal components, the values attached to the three variables were nearly equal; in the scaled first principal component the values were more or less the same. This indicates that in the first principal component the three shell variables play equal roles in shell selection. The coefficient of variation of the first and second principal components in the unscaled situation was very close to the

multiple regression equation. But in the scaled situation the coefficient of variation of second principal component was negligible.

4. Discussion

The first principal component seems to be a more reliable estimator of shell size for hermit crabs than selection by multiple regression of the best pair of shell and crab size correlates (Kuris and Brody 1976). In the present study also, both in scaled and unscaled conditions, the variability of the shell parameters could be mostly explained by the first principal component. Similar results were also reported by Mitchell (1976). When a multiple regression equation was calculated, with first principal component and second principal component against carapace length, the coefficient of variation of the two components in unscaled situation was more or less equal. In scaled condition, the coefficient of variation for first principal component was high and it was negligible in the case of second component. It therefore seems to be advisable to look at the scaled analysis and interpret the first principal component as the main guideline which influences *C. longitarsus* to select its shell. In *Pagurus bernhardus*, the percentage contributions to the total variability of the shell parameters of the first principal component was slightly less in the case of scaled analysis than in the unscaled analysis (Mitchell 1976). Even then, the percentage of variation in crab weight explained by the scaled first principal component was more than that of unscaled first principal component. But in the present study, the percentage contribution to the total variability of shell parameters was more in the scaled first principal component than in the unscaled first principal component. So it is probable that the scaled first principal component will explain more percentage of variation in crab length than the second principal component. This first principal component can be very easily interpreted, as, in all the cases, the length concerned with all the three shell variables is almost equal. This means that all the three shell variables play equally important roles in the selection of a shell.

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References

- Ajmal Khan S Mercy Thomas and Natarajan R 1980 Principal component analysis in the shell selection behaviour of the land hermit crab *Coenobita cavipes* Stimpson ; *Indian J. Mar. Sci.* 9 293-294
- Ajmal Khan S and Natarajan R 1981 Coexistence in hermit crabs of Vellar estuary ; *Indian J. Mar. Sci.* 10 201-204
- Bailey R T J (ed.) 1959 *Statistical methods in biology* (London : English Universities Press) p. 51
- Grant W C and Ulmer K M 1974 Shell selection and aggressive behaviour in two sympatric species of hermit crabs ; *Biol. Bull.* 146 32-43
- Kuris M A and Brody M S 1976 Use of principal component analysis to describe the snail shell resources for hermit crabs ; *J. Exp. Mar. Biol. Ecol.* 22 69-77
- Mitchell K A 1976 Shell selection in the hermit crab *Pagurus bernhardus* , *Mar. Biol.* 35 335-343
- Reese E S 1963 The behavioural mechanisms underlying shell selection by hermit crabs ; *Behaviour* 21 78-126

Evaluation of some organophosphorus insecticides against *Dacus cucurbitae* Coquillett on peach†

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Abstract Toxicity and persistence of fenitrothion, fenthion, malathion, methyl parathion, and trichlorophon applied at 0.05% (400 g/ha) were evaluated on peach fruits (*Prunus persica* L.) against the neonate larvae of *Dacus cucurbitae* Coquillett in two seasons (1977-78). Fenitrothion and methyl parathion were highly toxic materials followed by fenthion and malathion, while trichlorophon was the least toxic. Fenitrothion was highly persistent (12 days) followed by methyl parathion (7 days). All the insecticide residues were within the acceptable limits at the time of harvest.

Keywords. Organophosphorus insecticides ; toxicity ; persistence.

1. Introduction

Fruit fly, *Dacus cucurbitae* Coquillett, is a serious insect pest of peach (*Prunus persica* L.) in Himachal Pradesh. The crop sustains severe injuries by the larvae when the fruits are about to ripen and render these unfit for human consumption. There is a possibility of preventing the oviposition of the fruit fly on the peach fruits by giving a protective cover spray of an effective insecticide. Pruthi (1969), Myburgh (1961), Sampio *et al* (1966), Peretz *et al* (1966), Nagappan *et al* (1970), Anonymous, (1975) and Sharma *et al* (1973) reported that the pest could be controlled by a number of less persistent insecticides by such sprays. None of these reports are, however, based on detailed experimentation of intrinsic toxicity to the neonate larvae of fruit fly, persistence of effective toxicity or consumers' safety following their applications. Taking these as objectives in view, the present contribution reports the results of evaluation of the effectiveness of two spray schedules of fenitrothion, fenthion, malathion, methyl parathion and trichlorophon on peach against the neonate larvae of fruit fly.

† Part of Ph.D. thesis submitted to the Himachal Pradesh Krishi Vishva Vidyalaya, Palampur, by the senior author under the guidance of the second author.

2. Materials and methods

Commercial formulations of five organophosphorus (OP) insecticides viz. fenitrothion, fenthion methyl parathion, malathion and trichlorphon were sprayed on peach trees (cv 'Webecock') in an orchard of the Department of Horticulture, Himachal Pradesh Agricultural University, Palampur, with the help of a foot sprayer (Maruti make) to 'run-off' at the recommended rates of 0.05% concentration (400 g/ha). The experiment was conducted in a randomized block design taking single-tree plots for a replication. There were 18 trees for 5 treatments and a control which were replicated 3 times. The trees were 6-7 years of age with 6 m planting distance in a hexagonal system. All the other horticultural operations and fertilizer applications followed in the orchard were according to the recommendation of the package of practices for horticultural crops, Himachal Pradesh (Anonymous 1975). The trees were first sprayed on 21 May 1977 and again sprayed on 4 June 1977. Samples were collected at 0-day (immediately after the fruits were dried), 1, 3, 5, 7 and 14 days following treatments. The samples were later processed for estimating the deposits. The experiment was repeated with two sprays of the above insecticides at the same concentration in 1978 at the same location with another set of 18 trees having similar age and bearing. The first spray was given on 18 May 1978, and the second on 1 June 1978. The sampling intervals were same as in the first year.

Eight fruits were randomly collected from all around the periphery of each treated tree per treatment at each interval for residue analysis. Before extraction, the weight of the sample was recorded and the surface area of each fruit was determined by Turrell's (1946) method. The deposits of the respective fruit samples of each insecticide were extracted in redistilled solvents (benzene for fenitrothion, fenthion, methyl parathion and trichlorphon, and CCl_4 for malathion), by taking a sample of 8 fruits in a wide mouthed stoppered glass bottle (capacity 1L) and sufficient solvent was added so as to cover the fruits. Volume of the solvent was recorded and the bottle was labelled before shaking on a shaking machine for one hour. The extract was filtered into another container to which sufficient quantity of anhydrous sodium sulphate was added to remove the moisture and stored in a refrigerator until analysed. The extracts were then subjected to bio- and chemical assays. For bioassay (table 1), adult males of *Drosophila melanogaster* Meig. were taken as test insects and the rest of the procedure was the same as reported by Thakur and Hameed (1980). For chemical assays, the extracts of fenitrothion and methyl parathion were cleaned up by the method of Thornburg (1963); for malathion, Hameed and Rattan Lal's method (1971), and for fenthion and trichlorphon, the method of Jain *et al* (1974a) were used. Results of the quantitative estimations obtained by bioassay were verified by standard chemical methods. For fenitrothion and methyl parathion, the procedure of Averell and Norris (1948) was used and for malathion the method of Sutherland (1964) was used. Fenthion and trichlorphon residues were assayed by the method of Jain *et al* (1974a). Standard calibration curves were drawn separately for each insecticide. Recovery of each insecticide was studied in two different ways. Firstly, the insecticides were recovered from the surface deposits and secondly, as total residues. Insecticides were more satisfactorily recovered from the surface of the fruits than from the whole fruits (total residues). Fenitrothion recovered

Table 1. Dosage-mortality response of insecticides to *Drosophila melanogaster*.

Insecticide	Regression equation	LD ₅₀ (μg)	Fiducial limits
Fenitrothion	$Y = 1.6038x + 2.5246$	0.0035	0.0030 0.0041
Fenthion	$Y = 1.7825x + 1.6888$	0.0720	0.0624 0.0832
Malathion	$Y = 1.7758x + 0.7684$	0.2415	0.2098 0.2780
Methyl parathion	$Y = 1.8650x + 1.0484$	0.1315	0.1166 0.1482
Trichlorphon	$Y = 2.2240x + 0.9507$	0.4739	0.4204 0.5342

In none of these cases the data were found to be significantly heterogeneous at $P = 0.05$.

Y = Probit kill ; x = log conc. (μg/2ml) $\times 10^4$ for fenitrothion and 10^3 for rest of the insecticides.

to the extent of 85.9–89.6% (surface deposit) and 83.3–86.0% (total residue), fenthion 82.6–83.1% (surface deposit) and 82.5–83.5% (total residue), malathion 93.3–96.2% (surface deposit) and 90.3–91.7% (total residue), methyl parathion 92.3–93.3% (surface deposit) and 91.1–92.4% (total residue), and trichlorphon 80.2–80.8% (surface deposit) and 78.9–80.7% (total residue).

Peach fruits were harvested on 23 June 1977 and 23 June 1978 in the 1st and 2nd seasons respectively. Residues were later extracted by macerating them in a Waring blender with equal quantity of anhydrous sodium sulphate in a known volume of solvent. The slurry so obtained was decanted, filtered and cleaned up as per the method reported by Thakur and Hameed (1980). Intrinsic toxicity of the deposits of the 5 OP insecticides to the neonate larvae of fruit fly (table 3) was also determined by bioassay and the actual amount of insecticides in the deposits so formed from their commercial formulations, giving the desired toxicity, was determined chemically and by bioassay.

Half-life values of each insecticide on peach were worked out on the basis of the formula of Hoskins (1961). Safety interval (days) was determined on the basis of formula given by Thakur and Hameed (1980). Effective life of each insecticide was found out by substituting the value of log LD₅₀ to the Y of time deposit regression equations.

1. Results and discussion

Residue-film method of bioassay was very satisfactory (table 2) as it could detect residues of fenitrothion as low as $0.50 \mu\text{g}/\text{cm}^2 \times 10^{-3}$ compared with $416 \mu\text{g}/\text{cm}^2 \times 10^{-3}$ by the colorimetric method of Averell and Norris (1948). Fenitrothion

Table 2. Sensitivity of bio and chemical assays.

Insecticides	Bioassay $\mu\text{g}/\text{cm}^2 \times 10^{-2}$		Chemical assay $\mu\text{g}/\text{cm}^2 \times 10^{-3}$	
	Surface deposit	Total residue	Surface deposit	Total residue
Fenitrothion	0.5005	0.5160	399.9553	416.3180
Fenthion	16.6366	16.7454	714.6489	707.2002
Malathion	49.0888	49.9509	495.4271	527.6732
Methyl parathion	29.0868	29.4828	191.0425	193.0698
Trichlorphon	155.4194	159.3025	1052.4938	1046.2320

Table 3. Toxicity of insecticide deposits to the neonate larvae of *Dacus cucurbitae*.

Insecticide	Regression equation	LD ₅₀ ($\mu\text{g}/\text{cm}^2$)	Fiducial limits ($\mu\text{g}/\text{cm}^2$)	LD ₉₀ ($\mu\text{g}/\text{cm}^2$)	Fiducial limits ($\mu\text{g}/\text{cm}^2$)
Fenitrothion	$Y = 1.8837x + 1.4404$	0.0776	0.0594 0.1014	0.3715	0.2090 0.6758
Fenthion	$Y = 1.7465x + 1.2891$	0.1333	0.0997 0.1780	0.7219	0.3755 1.3868
Malathion	$Y = 1.4011x + 1.8860$	0.1669	0.1160 0.2403	1.3715	0.5777 3.2562
Methyl parathion	$Y = 1.4353x + 2.1853$	0.0914	0.0637 0.1312	0.7145	0.3455 1.4784
Trichlorphon	$Y = 1.7913x + 0.8080$	0.2189	0.1631 0.2938	1.1366	0.6112 2.1135

Y = Probit kill. In none of these cases the data were found to be significantly heterogeneous at $P = 0.05$.

$x = \log \text{conc.} \times 10^3 \mu\text{g}/\text{cm}^2$.

was a highly toxic material to the vinegar flies (table 1) which increased the sensitivity of the method. Fenitrothion and methyl parathion were also highly toxic (table 3) to the larvae of *D. cucurbitae* followed by fenthion and malathion, while trichlorphon was the least toxic material. Toxicity of deposits on the basis of minimum effective level (m.e.l.), i.e., LD₉₀ value (Gratwick and Tew 1966) were : fenitrothion > methyl parathion > fenitrothion > trichlorphon > malathion.

Table 4. Extent and magnitude of insecticide deposits in relation to their toxicity to the neonate larvae of *Dacus cucurbitae*.

Insecticide (at 0.05 % conc.)		Initial deposit ($\mu\text{g}/\text{cm}^2$)			
		First season 1977		Second season 1978	
		First spray (21-5-1977)	Second spray (4-6-1977)	First spray (18-5-1978)	Second spray (1-6-1978)
Fenitrothion	1.	6.570	6.967	7.404	7.748
	2.	84.66	89.78	95.41	99.84
	3.	17.68	18.75	19.93	20.85
		(1 : 5)	(1 : 5)	(1 : 5)	(1 : 5)
Fenthion	1.	7.105	7.011	7.205	7.356
	2.	53.30	52.59	54.05	55.18
	3.	9.84	9.71	9.98	10.19
		(1 : 5)	(1 : 5)	(1 : 5)	(1 : 5)
Malathion	1.	6.946	7.088	7.122	7.165
	2.	41.62	42.47	42.67	42.93
	3.	5.06	5.17	5.19	5.22
		(1 : 8)	(1 : 8)	(1 : 8)	(1 : 8)
Methyl parathion	1.	7.167	7.157	9.014	8.939
	2.	78.41	78.30	98.62	97.80
	3.	10.03	10.02	12.61	12.61
		(1 : 8)	(1 : 8)	(1 : 8)	(1 : 8)
Trichlorphon	1.	6.060	6.086	7.198	7.170
	2.	27.68	27.80	32.88	32.75
	3.	5.33	5.35	6.33	6.31
		(1 : 5)	(1 : 5)	(1 : 5)	(1 : 5)

1. Initial deposits, (average of bioassay and chemical assay).

2. Number of times the initial deposit exceeding the LD_{50} value *vide* table 3.

3. Number of times the initial deposit exceeding the m.e.l. (LD_{50}) value *vide* table 3.

Figures in parentheses are ratios between m.e.l. and intrinsic toxicity of deposits.

The results were in agreement with the findings of Hameed *et al* (1980). The deposits of all the insecticides in general were high, much in excess to their respective m.e.l. (table 4). The deposits of fenitrothion, for example, on peach fruits gave deposits of 6.57-7.75 $\mu\text{g}/\text{cm}^2$ (tables 6 and 7) in two sprays of the two seasons which were in excess of its m.e.l. (0.3715 $\mu\text{g}/\text{cm}^2$, table 3).

Similarly, the deposits of fenthion and methyl parathion were about 10-11 times in excess of their m.e.l.s. But the deposits of malathion and trichlorphon were minimum i.e. only about 5-6 times in excess of m.e.l.s. when compared with their respective LD_{50} values. The deposits of fenitrothion were far in excess (92 times) of their intrinsic toxicity (LD_{50}) followed by methyl parathion (86 times), fenthion (54 times), malathion (43 times) and trichlorphon (30 times). These observations showed that all these chemicals provided adequate deposits for the

Table 5. Threshold of toxic action of insecticide deposits to the neonate larvae of *Dacus cucurbitae*.

Insecticides recommended dose 0.05 (a.i.)	Deposits of insecticides on 14th day ($\mu\text{g}/\text{cm}^2$)			
	First season (1977)		Second season (1978)	
	First spray	Second spray	First spray	Second spray
Fenitrothion	0.224 (80.8)	0.267 (84.4)	0.300 (86.6)	0.288 (85.8)
Fenthion	0.062 (28.1)	0.052 (23.8)	0.111 (44.5)	0.121 (47.1)
Malathion*	0.108 (39.6)	0.159 (48.9)	0.146 (46.8)	0.202 (54.6)
Methyl parathion	0.102 (52.7)	0.095 (51.0)	0.115 (55.7)	0.113 (53.3)
Trichlorphon	0.049 (12.2)	0.038 (8.7)	0.066 (17.6)	0.056 (14.4)

* Deposits of insecticides on 7th day.

Figures in parentheses are corresponding probable kill of *Dacus cucurbitae* larvae vide regression equations given in table 3.

control of insects. Although the initial deposits of fenitrothion and methyl parathion in general were in excess of their intrinsic toxicity, the margin between m.e.l. and intrinsic toxicity (LD_{50}) of the deposits (mentioned in ratios) was the highest only with methyl parathion and malathion followed by fenitrothion, fenthion and trichlorphon. Thus, it can be concluded that the deposits of fenitrothion followed by methyl parathion provided comfortable margins when compared either with their LD_{90} or LD_{50} .

The residues of insecticides on the 14th day (tables 6 and 7) when subjected to the respective regression equations (table 3), corresponding per cent kill of the neonate larvae of fruit fly was obtained (table 5). The deposits of $0.30 \mu\text{g}/\text{cm}^2$ of fenitrothion in the 1st spray (2nd season) on the 14th day following treatment (table 5) gave 87% mortality of the larvae, followed by methyl parathion, fenthion and trichlorphon. Malathion, however, after 7 days did not afford more than 55% mortality of the insect.

Results of the field experiments on the persistence of 5 OP insecticides are summarized in tables 6 and 7. Chemical estimations in all the cases approximately agreed well with the data obtained from the bioassay of field-sample extracts. Data of the two estimations of each insecticide in both the seasons were also positively correlated to each other. During the 1st season, bioassay and chemical estimation of the deposits of 5 OP insecticides sprayed twice at an interval of 15 days on peach fruits showed the highest deposit with methyl parathion on 0-day (i.e. 7.17 and $7.16 \mu\text{g}/\text{cm}^2$) for the 1st and 2nd spray, respectively followed

Table 6. Persistence of insecticide deposits in peach fruits (first season, 1977), two sprays.

Insecticide	Method of estimation	No. of sprays	Deposit ($\mu\text{g}/\text{cm}^2$) following treatment at					
			0-day	1-day	3-day	5-day	7-day	14-day
Fenitrothion	Bioassay	I	6.46	2.64	1.89	0.87	0.58	0.21
	Chem. assay		6.68	2.84	1.82	0.86	0.60	0.24
	$r = 0.9994$		(6.57)	(2.74)	(1.85)	(0.86)	(0.59)	(0.22)
	Bioassay	II	6.94	2.64	1.73	0.83	0.69	0.28
	Chem. assay		6.99	2.69	1.89	0.79	0.60	0.25
	$r = 0.9995$		(6.97)	(2.66)	(1.81)	(0.81)	(0.64)	(0.27)
Fenthion	Bioassay	I	7.30	2.61	2.00	1.22	0.73	0.08
	Chem. assay		6.91	2.71	2.00	1.07	0.78	0.04
	$r = 0.9603$		(7.10)	(2.66)	(2.00)	(1.14)	(0.75)	(0.06)
	Bioassay	II	7.16	2.84	1.91	1.12	0.71	0.07
	Chem. assay		6.86	2.74	1.94	1.24	0.66	0.03
	$r = 0.9994$		(7.01)	(2.79)	(1.93)	(1.18)	(0.68)	(0.05)
Malathion	Bioassay	I	7.02	2.02	0.86	0.38	0.10	BDL
	Chem. assay		6.91	1.92	0.76	0.33	0.11	BDL
	$r = 0.9999$		(6.95)	(1.97)	(0.81)	(0.35)	(0.11)	
	Bioassay	II	7.12	1.81	0.85	0.37	0.16	BDL
	Chem. assay		7.06	1.73	0.91	0.38	0.15	BDL
	$r = 0.9998$		(7.09)	(1.77)	(0.88)	(0.38)	(0.16)	
Methyl parathion	Bioassay	I	7.13	3.38	2.35	1.04	0.79	0.11
	Chem. assay		7.21	3.31	2.29	1.08	0.79	0.09
	$r = 0.9998$		(7.17)	(3.34)	(2.32)	(1.06)	(0.79)	(0.10)
	Bioassay	II	7.18	3.07	2.12	0.86	0.63	0.09
	Chem. assay		7.13	3.10	2.23	0.85	0.64	0.09
	$r = 0.9998$		(7.16)	(3.09)	(2.18)	(0.85)	(0.63)	(0.09)
Trichlorphon	Bioassay	I	6.03	2.62	1.59	0.84	0.27	0.05
	Chem. assay		6.09	2.63	1.67	0.83	0.27	BDL
	$r = 0.9884$		(6.06)	(2.63)	(1.63)	(0.83)	(0.27)	(0.05)
	Bioassay	II	6.07	2.54	1.54	0.71	0.20	0.04
	Chem. assay		6.10	2.67	1.51	0.63	0.21	BDL
	$r = 0.9996$		(6.09)	(2.61)	(1.52)	(0.67)	(0.21)	(0.04)
Surface area (sq. cm) of one peach fruit**		I	30.59	31.31	32.06	32.67	34.13	36.60
		II	37.97	39.01	39.62	40.72	42.30	45.65
Percentage increase in fruit size over zero day sample		I	0	2.35	4.80	6.80	11.57	19.65
		II	24.12	27.52	29.49	33.11	38.28	49.23

 r = Coefficient of correlation significant at $P = 0.01$.

* Average of 3 replications

** Average of 120 fruits.

BDL = Below detectable limits

Figures in parentheses are average of bioassay and chemical assay

Average weather conditions	Temp. °C		RH	Rainfall (mm)
	Max.	Min.		
1st spray	29.93	20.79	55.35	3.73
2nd spray	27.40	18.89	61.90	3.32

Table 7. Persistence of insecticide deposits on peach fruits (second season, 1978) two sprays.

Insecticide	Method of estimation	No. of sprays	Deposits ($\mu\text{g}/\text{cm}^2$) following treatment at					
			0-day	1-day	3-day	5-day	7-day	14-day
Fenitrothion	Bioassay	I	7.42	2.86	1.85	1.10	0.83	0.30
	Chem. assay		7.39	2.97	1.83	1.04	0.84	0.30
	$r = 0.9998$		(7.40)	(2.91)	(1.84)	(1.07)	(0.84)	(0.30)
	Bioassay	II	7.67	2.88	1.76	1.08	0.85	0.28
	Chem. assay		7.83	3.02	1.91	1.05	0.77	0.30
	$r = 0.9995$		(7.75)	(2.95)	(1.83)	(1.06)	(0.81)	(0.29)
Fenthion	Bioassay	I	7.17	2.67	2.00	1.23	0.85	0.10
	Chem. assay		7.24	2.79	2.15	1.26	0.79	0.12
	$r = 0.9998$		(7.20)	(2.73)	(2.08)	(1.25)	(0.82)	(0.11)
	Bioassay	II	7.27	2.92	1.96	1.03	0.70	0.10
	Chem. assay		7.44	2.79	1.39	1.04	0.51	0.14
	$r = 0.9993$		(7.36)	(2.85)	(1.39)	(1.03)	(0.60)	(0.12)
Malathion	Bioassay	I	7.15	1.73	0.87	0.42	0.15	BDL
	Chem. assay		7.10	1.56	0.85	0.43	0.14	BDL
	$r = 0.9997$		(7.12)	(1.64)	(0.86)	(0.42)	(0.15)	..
	Bioassay	II	7.20	1.40	0.85	0.36	0.19	BDL
	Chem. assay		7.13	1.44	0.85	0.37	0.22	BDL
	$r = 0.9999$		(7.16)	(1.42)	(0.85)	(0.36)	(0.20)	
Methyl parathion	Bioassay	I	9.13	3.62	2.71	1.26	0.96	0.12
	Chem. assay		8.89	3.71	2.59	1.41	0.93	0.12
	$r = 0.9994$		(9.01)	(3.67)	(2.65)	(1.32)	(0.95)	(0.12)
	Bioassay	II	9.06	3.25	2.32	1.15	0.69	0.12
	Chem. assay		8.81	3.17	2.25	1.08	0.65	BDL
	$r = 0.9999$		(8.94)	(3.21)	(2.24)	(1.12)	(0.67)	(0.12)
Trichlorphon	Bioassay	I	7.22	2.81	1.97	1.24	0.43	0.07
	Chem. assay		7.17	2.87	2.07	1.30	0.47	0.06
	$r = 0.9998$		(7.20)	(2.84)	(2.02)	(1.27)	(0.45)	(0.07)
	Bioassay	II	7.20	2.65	1.91	1.06	0.35	0.06
	Chem. assay		7.14	2.87	1.88	1.13	0.33	0.05
	$r = 9992$		(7.17)	(2.76)	(1.89)	(1.10)	(0.34)	(0.06)
Surface area (sq. cm.) of one peach fruit**		I	25.13	27.21	27.88	28.69	29.96	31.21
		II	31.41	32.00	32.66	33.06	33.73	36.60
Percentage increase in fruit size over zero day sample		I	0	8.28	10.94	14.17	19.22	24.19
		II	24.99	27.34	29.96	31.56	34.22	45.64

r = Coefficient of correlation significant at $P = 0.01$

* Average of three replications

** Average of 120 fruits

BDL = Below detectable limits

Figures in parentheses are average of bioassay and chemical assay

Average weather conditions	Temp. °C		RH	Rainfall (mm)
	Max.	Min.		
1st spray	33.54	20.96	27.17	1.44
2nd spray	32.02	22.35	46.41	2.14

Table 8. Biological performance of insecticide on peach.

Insecticides (at 0.05% conc.)	Initial deposit in		Half life (days)	Effective life (days)	Harvest time residues (ppm)	Recommended tolerance level (ppm)	Safety interval (days)
	($\mu\text{g}/\text{cm}^2$)	(ppm)					
First season (1st spray) 1977							
Fenitrothion	6.570	12.898	3.1	10.35	..	0.50 ¹	14.47
Fenthion	7.105	13.987	2.3	5.95	..	0.50 ²	10.64
Malathion	6.946	13.239	1.3	2.15	..	6.00 ³	1.44
Methyl parathion	7.167	14.258	2.4	6.65	..	1.00 ⁴	9.25
Trichlorphon	6.060	12.620	2.1	4.00	..	0.205 ⁵	12.35
First season (2nd spray) 1977							
Fenitrothion	6.967	14.166	3.3	10.85	0.172	0.50 ¹	15.77
Fenthion	7.011	14.120	2.1	6.30	0.023	0.50 ²	10.21
Malathion	7.088	13.334	1.4	2.25	B.D.L.	6.00 ³	1.61
Methyl parathion	7.157	14.952	2.4	6.65	0.020	1.00 ⁴	9.25
Trichlorphon	6.086	12.525	1.9	3.35	0.136	0.205 ⁵	11.62
Second season (1st spray) 1978							
Fenitrothion	7.404	14.774	3.4	11.60	..	0.50 ¹	16.48
Fenthion	7.205	14.047	2.5	7.10	..	0.50 ²	12.20
Malathion	7.122	13.536	1.4	2.35	..	6.00 ³	1.65
Methyl parathion	9.014	17.771	2.4	7.30	..	1.00 ⁴	9.93
Trichlorphon	7.198	13.428	2.2	4.60	..	0.205 ⁵	13.17
Second season (2nd spray) 1978							
Fenitrothion	7.748	14.980	3.3	11.65	0.233	0.50 ¹	16.14
Fenthion	7.356	13.668	2.5	6.60	0.024	0.50 ²	12.12
Malathion	7.165	13.456	1.5	2.15	B.D.L.	6.00 ³	1.77
Methyl parathion	8.939	17.609	2.4	6.90	0.018	1.00 ⁴	9.90
Trichlorphon	7.170	13.284	2.1	4.30	0.188	0.205 ⁵	12.61

* Average of bio and chemical assays. Recommended tolerance level ¹ Anon. (1976), ² Anon. (1972), ³ Anon. (1970) ⁴ Anon. (1969) ⁵ Anon. (1976 b).
BDL = Below detectable limit.

by fenthion, malathion and fenitrothion. Trichlorphon gave the lowest deposit (i.e. $6.06 \mu\text{g}/\text{cm}^2$) for the 1st spray and $6.09 \mu\text{g}/\text{cm}^2$ for the 2nd spray (table 6). The deposits of all these insecticides dissipated quickly up to the 1st day and thereafter, the degradation was gradual. In the case of malathion, fast dissipation of the deposits occurred and no residue could be estimated after the 7th day following the treatment. The results were in agreement with the findings of Deshmukh and Singh (1975) and Singh (1977). The figures of average weather condition during this period are given in table 6. The deposits decreased as the ambient temperature and the size of the fruits increased.

In the second season (table 7) maximum initial deposit of $9.0 \mu\text{g}/\text{cm}^2$ (1st spray) and $8.94 \mu\text{g}/\text{cm}^2$, (2nd spray) were obtained with methyl parathion, which was followed by fenitrothion, fenthion, trichlorphon and malathion. The deposits were slightly more because of the absence of rain.

The relative persistence of 5 OP insecticides on peach expressed as half-life values, revealed that fenitrothion was a highly persistent insecticide. It also provided maximum period of protection against the peach fruit fly larvae (10–11 days) following either of the two sprays (table 8). Methyl parathion was found to be the next highly persistent insecticide giving adequate initial deposits and providing about a week's protection following each spray. This chemical therefore could be considered as the next best insecticide with a safety interval of 9 days. Comparing the biological performance of trichlorphon and malathion, trichlorphon was more persistent than malathion, but it was of little benefit owing to its low intrinsic toxicity to the fruit fly larvae. Safety interval of trichlorphon (12–13 days) was more than fenthion, malathion and methyl parathion, because of its low tolerance level fixed on peach fruits. Safety interval of 16–17 days was found for fenitrothion (table 8). Of the 5 OP insecticides, tested in the present investigation, fenitrothion (in two spray schedule) outclassed the rest of the insecticides, provided protection against the neonate larvae of fruit fly for about 12 days and the peach fruits were safe for consumption after 14–16 days following each spray. Insecticide residues at the time of harvest of peach in both the seasons were much below the acceptable tolerance limits.

References

- Anonymous 1969 Official FDA tolerance complete through 1969 ; *Natl. Agric. Chem. Ass. News* 28 (3) 10
- Anonymous 1970 Malathion. Pesticide residues in food. Report of the 1970 joint FAO/WHO meeting. FAO agricultural series No. 474, p. 33
- Anonymous 1972 Fenthion. Pesticide residues in food. Report of the 1971 joint FAO/WHO meeting. FAO agricultural series No. 88, p. 45
- Anonymous 1975 Package of practices for fruit crops of Himachal Pradesh *Publn. of Directorate of Extm. Edu. Agri. Compl.* p. 22–27
- Anonymous 1976a Fenitrothion. Recommended international maximum limits for pesticide residues. Secretariat of joint FAO/WHO food standards programme, Codex alimentarius commission, Rome 5th series p. 13
- Anonymous 1976b Trichlorphon. Recommended international maximum limits for pesticide residues. Secretariat of joint FAO/WHO food standards programme, Codex alimentarius commission, Rome 5th series p. 502

- Averell P R and Norris M V 1948 Estimation of small amounts of 0, 0-diethyl 0-*p*-nitrophenyl thiophosphate; *Anal. Chem.* **20** 753-6
- Deshmukh S N and Singh J 1975 Dissipation of carbaryl and malathion from okra fruits; *Indian J. Entomol.* **37** 64-7
- Gratwick M, Sillibourne J M and Tew R P 1965 The toxicity of insecticides to larvae of the codling moth, *Cydia pomonella* (1); *Bull. Entomol. Res.* **56** 367-88
- Gratwick M and Tew R P 1966 A comparison of the toxicity of various carbamate, organophosphorus and organo-chlorine compounds to the codling moth; *Proc. 3rd British Insecticide and Fungicide Conf.* (1965), Brighton, pp. 276-85
- Hameed S F and Rattan Lal 1971 Residues of malathion on cabbage, cauliflower and *knol khol* estimated by bioassay and chemical assay methods; *Indian J. Entomol.* **33** 326-7
- Hameed S F, Suri S M and Kahsyap N P 1980 Toxicity and persistence of residue of some organophosphorus insecticides in cucumber fruits for the control of fruit fly *Dacus cucurbitae*; *Indian J. Agric. Sci.* **50** 73-7
- Hoskins W M 1961 Mathematical treatment of loss of pesticide residues; *Pl. Prot. Bull. FAO* **9** 163-8
- Jain H K, Pandey S Y, Agnihotri N P and Dewan R S 1974a Rapid estimation of organophosphorus insecticides; *Indian J. Entomol.* **36** 145-8
- Jain H K, Pandey S Y, Agnihotri N P, Dewan R S, Saxena A N and Peshwani K M 1974b. Dissipation of phorate and disulfoton in rape seed crop (*Brassica campestris*); *Indian J. Pl. Prot.* **1** 37-42
- Myburgh A C 1961 Lebaycid as a cover spray for fruit fly control; *S. Afr. J. Agric. Sci.* **4** 615-21
- Nagappan K and others 1970 Insecticidal trials for the control of the melon fruit fly *Dacus cucurbitae* infesting snake gourd; *Madras Agric. J.* **57** 24
- Peretz I R, Gavrielith E, Gurewitch and Frankel H 1966 Trials in the control of the Mediterranean fruit fly *Ceratitidis capitata* with organophosphorus insecticides; *Israel J. Entomol.* **1** 155-65
- Pruthi H S 1969 p. 977. *Text book of agricultural entomology* Indian Council of Agricultural Research, New Delhi
- Sampaio A S, Rigitane O, Suplicyfilho N and Orlando A 1966 Tests on the control of fruit flies on peach by the application of new insecticides; *Biologica* **32** 213-6
- Sharma P L, Srivastava S and Dhaliwal HS 1973 Chemical control of peach fruit flies; *Pesticides* **7** 20-1
- Singh G 1977 Persistence of malathion and Leptophos (phosve) residues in/on okra *Abelmoschus esculentus* Moench fruits; *Thesis Abst. (Hau, Hissar)* **3** 157-8
- Sutherland G L 1964 Malathion in *Analytical method for pesticides, Plant Growth Regulators and Food Additives* (ed. G Zweig) (New York and London: Academic press) **2** 283-93
- Thakur A K and Hameed S F 1980 Biological performance of some organophosphorus insecticides against *Quadrastipidiotus perniciosus* Comstock on apple; *Proc. Indian Acad. Sci. (Anim. Sci.)* **89** 587-601
- Thornburg W W 1963 Extraction and clean-up procedures. In *analytical methods of pesticides, plant growth regulators and food additives* (ed. G Zweig) (New York and London: Academic Press) **1** 87-108
- Turrell F M 1946 Tables of surface and volumes of spheres of prolate and oblate spheroids and spheroidal coefficients (Univ. Calif: Berkeley) p. 153

Structure and chemical composition of the cuticle of *Cirolana fluviatilis*, *Sphaeroma walkeri* and *Sphaeroma terebrans*

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Abstract. A comparative study has been made of the cuticular organisation of isopod wood borer *Sphaeroma terebrans*, a fouler *Sphaeroma walkeri* and a free living isopod *Cirolana fluviatilis*. The cuticle of *S. terebrans* shows both structural and chemical peculiarities. In *S. walkeri*, the epicuticle contains fuchsinophilic protein and gives evidence of primary tanning. In *C. fluviatilis* the epicuticle is similar to that of other isopods.

Keywords. Cuticle ; structure and chemical composition ; *Sphaeroma terebrans* ; histochemistry.

1. Introduction

Although the cuticle of arthropods conforms to a basic pattern comprising of an inner procuticle formed of chitin-protein complex and an outer lipo-protein epicuticle, it shows a wide range of modifications in structure and chemical composition in different groups. Dennell (1947) observed that the abbreviation of tanning, occurrence of a two layered epicuticle and calcification of the cuticle of crustaceans may be related to their aquatic habitat and to the ready availability of calcium in their natural environment.

Earlier work on cuticle of isopods is more limited than on decapod cuticle. The structure and chemical composition of the cuticle of *Porcellio scaber*, *Ligia exotica*, *Armadillidium vulgare* and *Oniscus asellus* have been studied by George and Sheard (1954), Mary (1968), Lagarrigue (1970) and Mary and Krishnan (1974). It is known that there is a general conformity in structure and chemical composition to that of the cuticle of decapod crustaceans. A point of interest is that isopods unlike decapod crustaceans, have a number of adaptive devices for terrestrial life. It is of interest to investigate the nature of modifications in the cuticle structure and chemical composition relevant to their adaptation to semiterrestrial and terrestrial mode of life.

The Sphaeromatidae, which include wood borers and epifoulers, are presumably adapted for their mode of life as borers or as foulers. The nature of the adaptation of the cuticle structure and chemical composition is investigated by a comparative study of a typical borer like *Sphaeroma terebrans* with a closely

allied species *Sphaeroma walkeri* which is not a borer but shows a substratum affinity to submerged wood. The results were compared with the cuticular structure of a free living type, *Cirolana fluviatilis*.

2. Material and methods

Specimens of *S. walkeri* and *S. terebrans* were collected from Madras harbour by immersing timber panels in the sea. Specimens of *C. fluviatilis* were also collected from Madras harbour. The animals were maintained under laboratory conditions by changing the sea water every day.

For histological preparations of the cuticle, the material was fixed in 5% formaldehyde, decalcified in 3% glacial acetic acid or 3% EDTA and embedded in paraffin or celloidin. The stains used were Mallory's triple stain, Masson's trichrome stain and Heidenhain's haematoxylin (Mallory 1938; Pantin 1948; Lillie 1954). Histochemical tests were performed on frozen sections of the cuticle which were prepared by impregnating the specimens with 12½% and 25% gelatin solution and the blocks were hardened in 5% formaldehyde (Carleton and Leach 1938).

For detection of chitin, the tests used were Chitosan test (Campbell 1929) and Schulze test (Clark and Smith 1936). For sulphydryl and disulphide groups, tetrazolium test (Barnett and Saligman 1952), nitroprusside test and ferric ferri-cyanide test (Lillie 1954; Pearse 1968) were performed. To detect protein constituents the tests included xanthoproteic test, Millon's test (Pearse, 1968), Hg/nitrite test (Lison 1936) and biuret test (Fearon 1946). The presence of lipids was tested by treatment with dyes such as Sudan black B (Baker 1946; Lillie 1954). For detecting calcium, alkaline pyrogallon test (Lison 1936), alizarine red-S and Vonkossa's test (Lillie 1954) were employed.

3. Results

The cuticle of *Cirolana fluviatilis* varies in thickness in different regions from 10 to 30 μ . Sections passing through the tergite reveal two well defined regions in the cuticle corresponding to epicuticle and procuticle. An outer, thin homogeneous layer, 7 to 10 μ thick is different in appearance and colour from a thicker lamellated region which may be subdivided into three distinct layers in the intermoult stage.

The epicuticular nature of the outer thin part is confirmed by treatment with chlorated nitric acid which separates the epicuticle from the procuticle by the differential solubility of the two layers in this reagent. At this stage the epicuticle is not light yellow coloured; the procuticle is not distinguishable into sub-divisions. When stained with Mallory, the epicuticle may be divisible into two regions, an outer thin blue staining membrane and below it, a fuchsinophil region (figure 1). The two parts correspond to outer epicuticle and inner epicuticle of other arthropods. The procuticle stains uniformly blue in Mallory and green in Masson's stain. Tests for protein show that the inner epicuticle contains a protein containing phenyl groups (table 1). The protein in the procuticle on the other hand is negative to these tests but reacts positively to biuret test. In this respect the protein constituents of the cuticle conform to those reported in the cuticle of decapod crustaceans and insects (Dennell 1947; Wigglesworth 1948). A feature of the

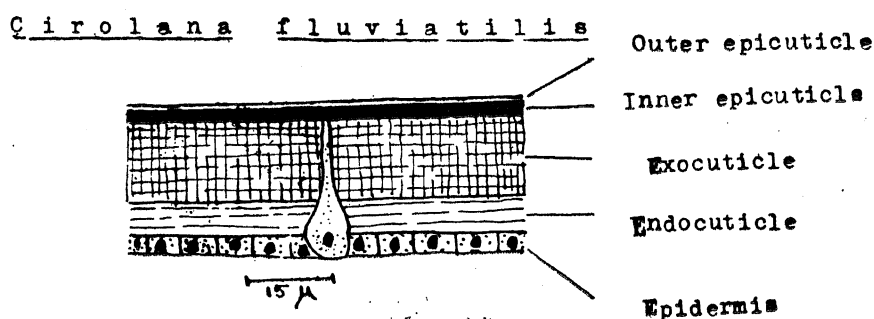


Figure 1. Transverse section through the intermoult cuticle, stained in Mallory's triple stain.

Table 1. Results of staining reactions and histochemical tests obtained with the late freshmoult cuticle of *Cirolana fluviatilis*.

No.	Stains and tests	References	Epicuticle		Procuticle
			Outer layer	Inner layer	
1.	Mallory's triple stain	Mallory 1938	Blue	Red	Blue
2.	Masson's trichrome stain	Trim 1941	Green	Red	Green
3.	Heidenhain's haematoxylin	Lillie 1954	Blue black	Grey	—
4.	Chitosan test	Campbell 1929	—	—	+
5.	Schultz modified test	Clark and Smith 1936	—	—	+
6.	Sudan Black B	Baker 1946	+	—	—
7.	Liebermann-Burchardt test	Lison 1953	+	—	—
8.	Biuret test	Fearon 1946	—	—	+
9.	Xanthoproteic test	Lillie 1954	—	+	—
10.	Millon's test	Pearse 1968	—	+	—
11.	Hg/nitrite test	Baker 1946	—	+	—
12.	Argentaffin test	Lison 1936	—	+	—
13.	Ferric chloride test	Lison 1936	—	+	—
14.	Blue tetrazolium test	Barnett and Seligman 1952	—	—	—
15.	Ferric ferricyanide test	Pearse 1968	—	—	—
16.	Alkaline pyrogallol test	Lison 1936	—	—	—
17.	Alizarin red-S	Lillie 1954	—	—	—
18.	Vonkossa's test	Lillie 1954	—	—	—

+ positive reaction ; — negative reaction.

protein of the cuticle of the isopod studied above, is the negative reaction to biuret test in the epicuticle which is positive to the Million and xanthoproteic tests.

The outer epicuticle reacts to tests for lipids and sterols. The inner epicuticle is only feebly reactive to these tests. It shows a positive reaction to argentaffin test which may be indicative of the presence of reducing substances which in the present context, considering this reaction together with the positive reaction obtained in the region with ferric chloride, may suggest that the reacting materials may be diphenols or polyphenols.

The structural features and staining reactions as well as the chemical composition of the cuticle differ in intermoult stage (figure 1). The epicuticular region in a section shows, an amber colouration and is unreactive to stains. The outer lipid epicuticle is less prominently seen in the sections. The procuticle is now distinguishable into an outer region which is amber colour and an inner region in which the lamellations are still clearly seen and still below is another region in which the lamellations are closely set. The results of histochemical tests are given in table 2. It is seen that the chemical composition of the epicuticle conforms to that in a number of decapod crustaceans in undergoing tanning resulting in acquisition of rigidity and resistance to chemical reagents.

In the procuticle prominent changes are brought about by the formation of an outer amber region giving rise to exocuticle and the part of the procuticle under it appears to be calcified and this region reacts to tests for calcium, like Vonkossa's test, alkaline pyrogallol and alizarin red-S. A region immediately below the calcified procuticle is free from calcium and is designated as the non-calcified layer. Results of tests applied for protein in the procuticle show that at this stage in addition to biuret positive protein and a protein involved in tanning, there is evidence of another protein which reacts positively to the blue tetrazolium and ferric ferricyanide tests. The presence of such a protein containing organic sulphur associated with calcified region has been earlier reported in decapod crustaceans like *Orconectes virilis* (Travis 1965). This author suggested that in the absence of tanning in this region the protein containing the SH group may play a role in facilitating calcification.

To examine how far the cuticular organisation of a closely allied fouler associated with wood differs from a free living form (described above) a detailed study of the cuticle of *S. walkeri* was made. Examinations of the stained and unstained sections of the cuticle of *S. walkeri* in the freshmoult condition showed epicuticle as in *Cirolana fluviatilis* distinguished by its homogeneity and in being formed of an outer thin membrane of the outer epicuticle (figure 2). The procuticle conforms in all respects to the condition reported in the corresponding stages of moult cycle of *Cirolana fluviatilis* (table 3). But in the intermoult stage there are seen marked differences in chemical features of the cuticle compared to those of intermoult cuticle of *Cirolana fluviatilis* (table 4). Unlike in *C. fluviatilis* the inner epicuticle does not undergo tanning. It however stains red in Mallory's and reacts positively to tests for protein like xanthoproteic and Million's. Similarly in the procuticle the outer part is not differentiated into an exocuticle but the middle region of the procuticle undergoes calcification and reacts to tests for calcium like Vonkossa's alizarin red-S and alkaline pyrogallol tests (table 4). From a comparative study of the intensity of the reaction to tests for calcium it may appear that calcium content is more than what was noted in the allied type. The region of the pro-

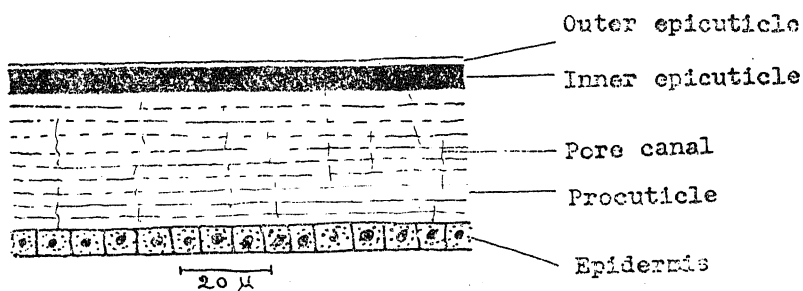
Sphaeroma walker

Figure 2. Transverse section through the freshmoult cuticle, stained in Mallory's triple stain.

Table 2. Results of staining reactions and histochemical tests obtained with the intermoult cuticle of *Cirolana fluviatillis*.

No.	Stains and tests	Reference	Epicuticle			Procuticle	
			Outer layer	Inner layer	Exocuticle	Calcified layer	Uncalcified layer
1.	Mallory's triple stain	Mallory 1938	Blue	Amber	Amber	Blue	Light blue
2.	Masson's trichrome stain	Trim 1941	Green	Amber	Amber	Green	Light blue
3.	Heidenhain's haematoxylin	Lillie 1954	Blue back	Grey	—	—	—
4.	Chitosan test	Campbell 1929	—	—	+	+	+
5.	Schultz modified test	Clark and Smith 1936	—	—	+	+	+
6.	Sudan Black B	Baker 1946	++	+	—	—	—
7.	Liebermann Burchardt	Lison 1953	++	+	—	—	—
8.	Biuret test	Fearon 1946	—	—	—	+	+
9.	Xanthoproteic test	Lillie 1954	—	+	+	—	—
10.	Millon's test	Pearse 1968	—	+	+	—	—
11.	Hg/nitrite test	Baker 1946	—	+	+	—	—
12.	Argentaffin test	Lison 1936	—	+	+	—	—
13.	Ferric chloride test	Lison 1936	—	—	—	—	—
14.	Blue tetrazolium test	Barnett and Seligman 1952	—	—	+	+	—
15.	Ferric ferricyanide test	Pearse 1968	—	—	+	+	—
16.	Alkaline pyrogallol test	Lison 1936	—	—	+	+	—
17.	Alizarin red-S	Lillie 1954	—	—	+	+	—
18.	Vonkossa's test	Lillie 1954	—	—	+	+	—

+ positive reaction ; ++ intensely positive ; — negative reaction.

cuticle reacting to calcium test is also abbreviated. In other respects it recalls the condition noted in the cuticle of *C. fluviatilis*. In the cuticle of *S. terebrans* which is a borer, the epicuticle shows a further deviation from the condition reported in *C. fluviatilis* (figure 3). These differences refer to the protein compound of the epicuticle which unlike in *S. walkeri* is not the fuchsinophil tyrosine containing protein. There is evidence of only the basal protein which is biuret positive, stains blue with Mallory's and green in Masson's stain. Complete absence of tanning is a feature of the epicuticle of this animal in all the stages of moult cycle (tables 5, 6).

Table 3. Results of staining reactions and histochemical tests obtained with the late freshmoult cuticle of *Sphaeroma walkeri*.

No.	Stains and tests	References	Epicuticle		Procuticle
			Outer layer	Inner layer	
1.	Mallory's triple stain	Mallory 1938	Blue	Red	Blue
2.	Masson's trichrome stain	Trim 1941	Green	Red	Green
3.	Heidenbain's haematoxylin	Lillie 1954	Blue black	Grey	—
4.	Chitosan test	Campbell 1929	—	—	+
5.	Schultz modified test	Clark and Smith 1936	—	—	+
6.	Sudan black B	Baker 1946	+	—	—
7.	Liebermann Burchardt	Lison 1953	+	—	—
8.	Biuret test	Fearon 1946	—	—	+
9.	Xanthoproteic test	Lillie 1954	—	+	—
10.	Millon's test	Pearse 1968	—	+	—
11.	Hg/nitrite test	Baker 1946	—	+	—
12.	Argentaffin test	Lison 1936	—	—	—
13.	Ferric chloride test	Lison 1936	—	—	—
14.	Blue tetrazolium test	Barnett and Seligman 1952	—	—	—
15.	Ferric ferricyanide test	Pearse 1968	—	—	—
16.	Alkaline pyrogallol test	Lison 1936	—	—	—
17.	Alizarin red-S	Lillie 1954	—	—	—
18.	Vonkossa's test	Lillie 1954	—	—	—

+ positive reaction ; — negative reaction.

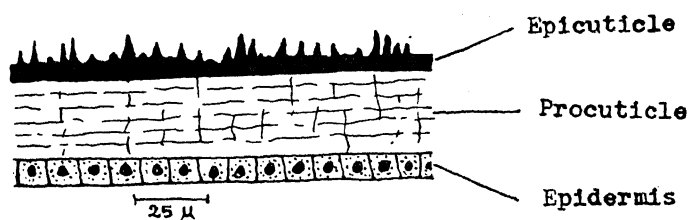
Sphaeroma terebrans

Figure 3. Transverse section through the freshmoult cuticle, stained in Mallory's triple stain.

Table 4. Results of staining reactions and histochemical tests obtained with the intermoult cuticle of *Sphaerome walkeri*.

No.	Stains and tests	References	Epicuticle		Procuticle	
			Outer layer	Inner layer	Calci-fied layer	Uncalci's fied layer
1.	Mallory's triple stain	Mallory 1938	Blue	Red	Blue	Light blue
2.	Masson's trichrome stain	Trim 1941	Green	Red	Green	Light blue
3.	Heidenhain's haematoxylin	Lillie 1954	Blue black	Grey	—	—
4.	Chitosan test	Campbell 1929	—	—	+	+
5.	Schultz modified test	Clark and Smith 1936	—	—	+	+
6.	Sudan black B	Baker 1946	+	—	—	—
7.	Liebermann-Burchardt test	Lison 1953	+	—	—	—
8.	Biuret test	Fearon 1946	—	—	—	+
9.	Xanthoproteic test	Lillie 1954	—	+	—	—
10.	Millon's test	Pearse 1968	—	+	—	—
11.	Hg/nitrite test	Baker 1946	—	+	—	—
12.	Argentaffin test	Lison 1936	—	—	—	—
13.	Ferric chloride test	Lison 1936	—	—	—	—
14.	Blue tetrazolium test	Barnett and Seligman 1952	—	—	+	—
15.	Ferric ferricyanide test	Pearse 1968	—	—	+	—
16.	Alkaline pyrogallol	Lison 1936	—	—	+	—
17.	Alizarin red-S	Lillie 1954	—	—	+	—
18.	Vonkossa's test	Lillie 1954	—	—	+	—

+ positive reaction - negative reaction

Table 5. Results of staining reactions and histochemical tests obtained with the freshmoult cuticle of *Sphaeroma terebrans*.

No.	Stain and tests	References	Epicuticle	Procuticle
1.	Mallory's triple stain	Mallory 1938	Blue	Light blue
2.	Masson's trichrome stain	Trim 1941	Green	Light green
3.	Heidenhain's hasmotaoxilin	Lillie 1954	Light blue	—
4.	Chitosan test	Campbell 1929	—	+
5.	Schultz modified test	Clark and Smith 1936	—	+
6.	Sudan Black B	Baker 1946	—	—
7.	Liebermann-Burchardt test	Lison 1953	—	—
8.	Biuret test	Fearon 1946	+	+
9.	Xanthoproteic test	Lillie 1954	—	—
10.	Millon's test	Pearse 1968	—	—
11.	Hg/nitrite test	Baker 1946	—	—
12.	Argentaffin test	Lison 1936	—	—
13.	Ferric chloride test	Lison 1936	—	—
14.	Blue tetrazolium test	Barnett and Seligman 1952	—	—
15.	Ferric ferricyanide test	Pearse 1968	—	—
16.	Alkaline pyrogallol	Lison 1936	—	—
17.	Alizarin red-S	Lillie 1954	—	—
18.	Vonkossa's test	Lillie 1954	—	—

+ positive reaction ; — negative reaction.

In the absence of a tyrosine containing protein which is the precursor of tanning of the cuticle, *S. terebrans* may be said to lack the essential mechanism for tanning. In the procuticle also there are seen marked deviations from the condition noted in *C. fluviatilis*. This refers to the non-differentiation of outer part of the procuticle into an exocuticle or a mesocuticle. Although there is evidence of calcification, compared to the other two types studied, it is much abbreviated. The results of tests are recorded in table 6.

4. Discussion

It deserves to be noted here that the cuticle of the wood borer *S. terebrans* is devoid of the outer epicuticle while those of *S. walkeri* which is a fouler, and of the free living *C. fluviatilis*, have this layer. The outer epicuticle is formed of lipid and is believed to check evaporation of water from the surface of the body (Beament 1961, 1964). Recent work has shown that the cuticle lining the gut in decapod crustacean like *Ocypoda platytarsis* lacks an outer lipid epicuticle, which accounts for the increased permeability to water through the layer (Mary and Krishnan 1974). The significance of the absence of an outer epicuticle in the wood

Table 6. Results of staining reactions and histochemical tests obtained with the intermoult cuticle of *Sphaeroma terebrans*.

No.	Stains and tests	References	Epicuticle	Procuticle
1.	Mallory's triple stain	Mallory 1938	Blue	Light blue
2.	Masson's trichrome stain	Trim 1941	Green	Light green
3.	Heidenhain's haematoxylin	Lillie 1954	Light blue	—
4.	Chitosan test	Campbell 1929	—	+
5.	Schultz modified test	Clark and Smith 1936	—	..
6.	Sudan Black B	Baker 1946	+	—
7.	Liebermann Burchardt test	Lison 1953	—	—
8.	Biuret test	Fearon 1946	+	..
9.	Xanthoproteic test	Lillie 1954	—	—
10.	Millon's test	Pearse 1968	—	—
11.	Hg/nitrite test	Baker 1946	—	—
12.	Argentaffin test	Lison 1936	—	—
13.	Ferric chloride test	Lison 1936	—	—
14.	Blue tetrazolium test	Barnett and Seligman 1952	—	+
15.	Ferric ferricyanide test	Pearse 1968	—	+
16.	Alkaline pyrogallol	Lison 1936	—	..
17.	Alizarin ed-S	Lillie 1954	—	..
18.	Vonkossa's test	Lillie 1954	—	..

+ positive reaction ; — negative reaction.

borer *Sphaeroma terebrans* may be that the cuticle in it is more permeable than that of the closely allied species *Sphaeroma walkeri*. This species in its natural habitat within the wood may not be exposed to fluctuations in ambient temperatures, to need protective devices against water loss. Similarly the absence of an outer epicuticle which would restrict the permeability to water and possibly ions, may not be an important and a necessary factor as the borer unlike the free living forms living within a restricted environment.

The wood boring species *S. terebrans* is characterized by the occurrence of spines on the cuticular surface. Spines are absent in *S. walkeri*. It thus appears that the presence of cuticular spines is somehow related with boring habit. The precise functional role and the spines in boring is not known.

The inner epicuticle which may undergo tanning is important in bringing about a restraint on water loss. In the wood borer *S. terebrans* the protein composition of the cuticle is very different from the allied species *S. walkeri* and *C. fluvialis* in the absence of the fuchsinophil protein and the presence of only a biuret positive protein in the epicuticle. This is an unusual feature in an intermoult cuticle. Immediately after moulting or in preecdysial cuticle it has been reported that the epicuticle may contain only a biuret positive protein which is seen over-

laid by a fuchsinophil protein which is a precursor of tanning (Dennell and Malek 1955). Tanned protein and precursors of tanning are known to prevent water loss (Sundararajulu and Krishnan 1968; Mary 1968).

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References

- Baker J R 1946 The histochemical recognition of lipine ; *Q. J. Micr. Sci.* **87** 441-471
- Barnett R J and Seligman A M 1952 Demonstration of protein bound sulphhydryl and disulphide groups by two new histochemical methods ; *J. Natl. Cancer Inst.* **13** 215-216
- Beament J W L 1961 The water relations of insect cuticle ; *Biol. Rev.* **36** 281-320
- Beament J W L 1964 *Advances in insect physiology* Vol. II (London and New York : Academic Press)
- Campbell F L 1929 The detection and estimation of chitin and the relations of chitinization to hardness and pigmentation of the American cockroach *Periplaneta americana* ; *Ann. Ent. Soc. America* **22** 401-426
- Clark G L and Smith A F 1936 X-ray diffraction studies on chitin, chitosean and derivatives ; *J. Phys. Chem.* **40** 863-879
- Carleton H M and Leach E H 1938 *Histological technique* (London : Oxford University Press) p. 383
- Charniaux-Legendre J 1951 Le cycle d'intermue des amphipodes et ses particularités chez les formes terrestres (Talitridae) ; *Arch. Zool. Exp.* **88** 178-204
- Dennell R 1947 The occurrence and significance of phenolic hardening in the newly formed cuticle of crustacea Decapoda ; *Proc. R. Soc.* **B134** 485-503
- Dennell R and Malek S R A 1955 The cuticle of cockroach *Periplaneta americana* III. The hardening of the cuticle impregnation preparatory to phenolic tanning ; *Proc. R. Soc.* **B143** 414-426
- Fearon M R 1946 *An introduction to biochemistry* (London : William Heinmann)
- George R W and Sheard K 1954 Ecdysis in the isopod *Porcellio scaber* (Latreille) ; *Aust. J. Zool.* **2** 75-85
- Lagarigue J Q 1970 Recherches ecophysiologiques sur les oniscoides (Isopodes Terrestres) ; (Université de Montpellier)
- Lillie R D 1954 *Histopathologic Technique and practical histochemistry* (New York : Blackiston) p. 501
- Lison L 1936 *Histochemie animale* (Paris : Gauthier-Villars) p. 534
- Lison L 1953 *Histochemie et Cytochemie animales*, Gauthier Villars, Paris.
- Mellory F B 1938 *Pathological technique* (Philadelphia : Saunders)
- Mary F 1968 Studies on the integument of some invertebrates in relation to regulation of body fluid concentration Ph.D. Thesis, Madras University.
- Mary F and Krishnan G 1974 On the nature and role of protein constituents of the cuticle of crustaceans in relation to permeability of the cuticle ; *Mar. Biol. (Berlin)* **125** 229-309
- Pantin G F A 1948 *Microscopic technique for zoologists* (London : Cambridge University Press)
- Pearse A G E 1968 *Histochemistry, theoretical and applied* (London : Churchill) p. 759
- Sundararajulu G and Krishnan G 1968 The epicuticle of millipedes belonging to the genera *Cingalobolus* and *Aulacobolus* with special references to seasonal variations ; *2. Marur* **23** 845-851
- Travis D F 1965 The deposition of skeletal structures in the Crustacea. 5. The histomorphological and histochemical changes associated with the development and calcification of the branchial exoskeleton in the crayfish, *Orconectes virilis* ; *Hagen Acta Histochem.* **20** 193-222
- Trim A R H 1941 Studies on the chemistry of the insect cuticle. 1. Some general observations on certain arthropod cuticles with special reference to the characterization of the proteins ; *Biochem. J.* **35** 1088-1098
- Wigglesworth V B 1948 The insect cuticle ; *Biol. Rev.* **C23** 408-451

Effect of some antibiotic compounds in cotton on post-embryonic development of spotted bollworm (*Earias vittella* F.) and the mechanism of resistance in *Gossypium arboreum*

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Abstract. Larval survival and post-embryonic development of the spotted bollworm, *Earias vittella* was studied on 23 genotypes belonging to three cultivated species of cotton (*Gossypium arboreum*, *G. barbadense* and *G. hirsutum*). There were significant differences in larval survival and post-embryonic development on different genotypes. The larval survival varied from 27.1 to 93.3%, developmental period from 16.6 to 20.3 days, pupation from 60 to 100% and adult emergence from 78 to 94%. Gossypol increased the post-embryonic developmental period. Majority of the larvae entered the bolls through the thallic region, possibly, to avoid higher concentrations of gossypol in the pericarpic region. Tannin content of bolls was significantly and negatively correlated with adult emergence.

Crosses between resistant and susceptible genotypes of *G. arboreum* segregated into pigmented (red) and non-pigmented (green) plant types. The former were rich in gossypol and tannins compared to the latter. Gossypol and tannin content of bolls showed negative correlation with spotted bollworm incidence.

Keywords. *Gossypium* ; gossypol ; tannins ; spotted bollworm ; *Earias* ; antibiotic ; post-embryonic ; resistance.

1. Introduction

Cotton is an important commercial crop in Asia, Africa, America and Australia. It is damaged by over 130 different species of insect pests. Spotted bollworms (*Earias* spp.) cause serious losses to cotton in India, China, Southeast Asia, Iraq, Israel and Africa (Sohi 1964; Chang *et al* 1963; Walker 1952; Avidov and Harpaz 1969; Reed 1974). Pigment glands characterizing genus *Gossypium* (Gillham 1965) had been identified as a source of resistance against the insects feeding on cotton plant (Bottger *et al* 1964). Antibiosis, as one of the mechanisms of resistance in cotton was first demonstrated by Brazzel and Martin (1956, 1959) in *G. tomentosum* against *Pectinophora gossypiella*. Later, antibiosis was reported against *Heliothis zea* and *H. virescens* (Lukefahr *et al* 1966; Lukefahr and Houghtaling 1969; Oliver *et al* 1970, 1971; Lukefahr *et al* 1974, 1975; Meisner *et al* 1977); *Anthonomus grandis* (Douglas 1966, Bailey *et al* 1967) and *Amrasca*

biguttula biguttula (Chakravarty and Sahni 1972). Gossypol (Lukefahr and Houghtaling 1966; Lukefahr *et al* 1966, 1975; Meisner *et al* 1977), *p-hemigossypolone* (Gray *et al* 1976), heliocides (Stipanovic *et al* 1976, 1977) and a condensed tannin (Chan and Waiss Jr. 1978) have been reported to confer resistance to insects feeding on cotton. Considering the colossal losses caused by this pest *vis-a-vis* the limitations of insecticides to control it, the host plant resistance can be used as one of the mechanisms to keep its populations at a low level. Asiatic diploid species (*Gossypium arboreum* L.) have been reported to be comparatively less damaged (Hussain and Khan 1940; Butani 1974). Singh *et al* (1972, 1976) found that within *G. arboreum*, damage by bollworms varied among different varieties and that resistance was genetically inherited. The present studies report the extent of antibiosis and the antibiotic factors affecting development of *E. vittella* in different cotton genotypes, and the mechanism of resistance in *G. arboreum*.

2. Materials and methods

The insect culture was raised in the laboratory on green cotton bolls of Bikaneri Nerma. Genotypes tested included 3 lines from *G. arboreum* (Sanguineum, Virnar and G-27), one from *G. barbadense* (Line 199-5) and 19 from *G. hirsutum*. The effect of antibiotic factors was studied on the survival of first instar larvae and the post-embryonic development. Larval survival on bolls of different genotypes was studied by releasing newly hatched larvae on green bolls (7-10 days old) in plastic boxes (15 × 15 × 5 cm). Five larvae were released on each boll. The plastic petridishes were kept inside B.O.D. incubator at 30 ± 1° C. Three days after inoculation, the bolls were dissected and the number of survivors recorded. The observations were made on 20 bolls of each genotype arranged in four sets and also on the number of larvae entering the boll through the thallic and pericarpic regions of the boll on a few genotypes.

The post-embryonic development was studied on 7-10 days old bolls of different genotypes. Food was changed every third day. The rearing was carried out at 30 ± 1° C. Observations were recorded on pupation, adult emergence, pupal weight, and larval and pupal developmental periods. Growth indices on different genotypes were calculated by the following formulae:

$$\text{Larval growth index (LGI)} = \frac{\text{Percent pupation}}{\text{Larval period (days)}}$$

$$\text{Total developmental growth index (TGI)} = \frac{\text{Percent emergence}}{\text{Total developmental period (days)}}$$

Spotted bollworm incidence was recorded on 100 green bolls during the peak activity period (August) in 1978 on five varieties of *G. arboreum* and three F₂ populations of intra-*arboreum* crosses involving resistant and susceptible types. The different genotypes were grown in 2 row-4 m plots. For chemical analysis, 10-15 days old bolls were collected. The bolls were dried at 40° C and powdered finely in a grinder. The gossypol content was determined by the method of Yang and Davis (1976) and tannins were estimated by indigocarmine volumetric method

(AOAC, 1975). The gossypol and tannins were expressed as per cent of dry weight of the sample taken. The data were analysed and simple correlations between the different parameters were worked out.

3. Results and discussion

The larval survival varied from 27.1 to 93.3% in different genotypes (table 1). Larval survival was minimal on SH-269, SS-265, Acala, Cocker-100A, Sanguineum, XG-15 and South Carolina. Only 27.1 to 40.7 per cent larvae survived on these lines. Comparatively, more larvae survived on PS-10, Virnar, RS-89 and 320-F (68.8 to 93.3%). This difference in survival values in different genotypes is indicative of the resistance offered by bolls of some genotypes. Larvae showed a tendency to enter the bolls through the thallic region (table 2), possibly, to avoid higher concentrations of antibiotic factors in the pericarpic region.

Table 1. Survival of first instar larvae and pupal weights of spotted bollworm (*E. vittella*) on the bolls, and amounts of gossypol and tannins in different varieties.

Variety	Larval survival (%)	Gossypol (%)	Tannins (%)	Pupal Weight (mg.)
PS-10	93.3	0.96	1.42	48.2
Virnar	76.0	0.76	1.60	66.9
320F	70.0	1.10	1.40	..
RS-89	68.7	1.04	1.40	52.4
D-33	60.4	1.12	1.35	55.3
JR-81	58.1	0.79	1.06	52.3
Stort-73IN	51.6	0.79	1.63	..
Bikaneri norma	48.8	0.92	1.22	..
Frego bract	48.3	1.00	1.49	..
M-495	47.3	1.04	1.58	50.3
H-14	47.0	1.08	1.87	43.7
<i>G. barbadense</i>	46.0	1.31	..	65.6
HR-26 # 8 X H.HG-6-1M	44.8	0.95	1.88	56.0
Hindiweed	43.3	1.13	1.60	46.3
BJR	42.0	0.98	1.57	..
XG-15	40.6	1.33	1.87	45.1
South Carolina	40.6	1.11	1.15	58.5
SH-269	40.3	1.02	1.60	42.3
SS-265	40.2	1.02	1.34	57.4
Acala	39.3	0.58	1.30	56.0
Cocker-100A	34.6	0.61	1.61	45.2
Sanguineum	27.1	1.46	1.96	57.2
C.D. at 5% t	13.22	0.13	0.04	10.1

($r = -0.7638$). High concentrations of gossypol in the pericarpic region possibly deterred the larvae entering the boll through this region.

Larval period was prolonged by gossypol and tannins. Total period required for completing post-embryonic development showed a positive and significant correlation with gossypol content of bolls ($r = 0.4974$) (table 5). The shorter developmental period on Vinnar and Empire was possibly due to lower amounts of gossypol in these genotypes, while the longer developmental periods on Sangre de Torro, SS-265, XG-15, GH-27 and *G. barbadense* could be attributed to higher concentrations of gossypol in these varieties. Similar antibiotic factors in cotton have been reported against *P. gossypiella* (Brazzel and Martin 1956; 1959); *Heliothis* spp. (Oliver *et al* 1970, 1971); *H. virescens* (Lukafahr *et al* 1966; Meisner *et al* 1977) and *Anthonomus grandis* (Bailey *et al* 1967; Douglas 1966).

Gossypol seemed to affect pupation and adult emergence adversely, though the correlation coefficients were very low (tables 6, 7). Tannins showed significant and negative correlations with adult emergence ($r = -0.7813$) and growth index ($r = -0.7432$) (table 7). The regression coefficients were also significant and negative.

Gossypol incorporated in artificial diet had been found to affect the growth and survival of pink bollworm (Shaver and Parrot 1970). The larval weight had been found to be negatively correlated with gossypol content (Wilson and Shaver 1973).

Some biochemical components other than gossypol and tannins also accounted for the antibiosis expressed by different genotypes. Eagle *et al* (1950) found no correlation between cotton seed pigment glands, toxicity and extractable gossypol. Some growth inhibiting factors have recently been reported in the race stocks of *G. hirsutum*, which contained medium amounts of gossypol but were highly antibiotic against *P. gossypiella* and *Heliothis* spp. (Lukafahr *et al* 1974). These additional growth inhibiting factors were later identified as *p*-hemigossypolone (Gray *et al* 1976) and Helicoides H₁ and H₂ (Stipanovic *et al* 1976, 1977). In the present studies, some factors other than gossypol also accounted for the antibiotic effect against *E. vittella*. Major role among these factors was that of tannins. Elligar *et al* (1978) investigated the toxicity and relative importance of other terpenoids in the pigment glands and suggested that these might supplement the toxicity of gossypol but themselves are of minor importance. Gossypol reduces the nutritional quality of boll contents (Carter and Lyman, 1969; Lyman *et al* 1959). It also inhibits the activity of enzymes protease, amylase and pepsinogen (Meisner *et al* 1978; Tanksley *et al* 1970). Antibiotic effects of gossypol are possibly due to reduced nutritional quality or non-availability of nutrients or enzyme inhibition, which lead to the prolonged development and reduced growth of the insect.

Bollworm incidence on different varieties and F₂ populations of intervarietal crosses have been presented in table 8. It was observed that Daulat, CJ-73 and non-pigmented F₂ segregates had > 75% spotted bollworm incidence compared to Lohit, G-27 and pigmented F₂ segregates which manifested < 55% incidence. The red pigmented segregates showed 32.42-46.87% incidence whereas non-pigmented (green) segregates had 62.50-80.06% bollworm incidence.

There were significant differences among different genotypes in gossypol and tannin content (table 9). The less susceptible genotypes viz., G-27 and Lohit as well as pigmented F₂ segregates had comparatively higher gossypol content

Table 5. Correlations between gossypol and tannin content in bolls with post embryonic development of *E. vittella*.

	Gossypol	Tannins	Larval Period	Pupal period	Total developmental period
Gossypol	1.000				
Tannins	0.2397	1.000			
Larval period	0.3398	0.3480	1.000		
Pupal period	0.1724	-0.0725	0.0519	1.000	
Total developmental period	0.4974*	0.1491	0.7364*	0.5242*	1.000
	b_i	t value	R^2		
Y_1 Larval period					
X_1 Gossypol	0.7239	1.1745			
X_2 Tannins	0.7743	1.2208	0.1908		
Y_2 Pupal period					
X_1 Gossypol	0.4473	0.7992			
X_2 Tannins	-0.2760	0.4793	0.0434		
Y_3 Total developmental period					
X_1 Gossypol	1.9673	2.1937*			
X_2 Tannins	0.1311	0.1421	0.2483		

* Significant at $P = 0.05$

Table 6. Relationship between gossypol and tannin content of bolls with pupation and growth index.

	% Gossypol	% Tannin	% Pupation	Growth index
% Gossypol	1.000			
% Tannin	0.5095	1.000		
% Pupation	-0.2485	-0.0148	1.000	
Growth index	-0.0359	-0.0131	0.9825*	1.000
	b_i	t value	R^2	
Y_1 Pupation				
X_1 Gossypol	-19.1455	0.8255		
X_2 Tannins	8.2009	0.3830	0.786	
Y_2 Growth index				
X_1 Gossypol	-3.8546	1.2135		
X_2 Tannins	1.7104	0.5835	0.1556	

Table 7. Relationship between gossypol and tannin content in bolls to per cent emergence and growth index.

	Gossypol	Tannins	Emergence	Growth index
Gossypol	1.000			
Tannins	0.4605	1.000		
Emergence	-0.3510	-0.7813*	1.000	
Growth index	-0.3411	-0.7432*	0.9198*	1.000
	b_i	r value	R^2	
Y_1 Emergence				
X_1 Gossypol	0.3281	0.0446		
X_2 Tannins	-17.1281	3.1635*	0.6104	
Y_2 Growth index				
X_1 Gossypol	0.0027	0.0052		
X_2 Tannins	-1.0993	2.7913*	0.5523	

* Significant at $p = 0.05$ Table 8. Incidence of spotted bollworm (*E. vittella*) on *arboresum* genotypes.

Sl. No.	Genotype	Incidence/ 100 bolls
1.	Daulat	90.00
2.	CJ 73	75.00
3.	Cernuum	69.20
4.	Lohit	55.00
5.	G-27	53.00
6.	Cernuum \times Lohit	
	(a) Non-pigmented F_2	80.00
	(b) Pigmented F_2	46.67
7.	Daulat \times Lohit	
	Non-pigmented F_2	80.00
	Pigmented F_2	45.45
8.	Cernuum \times G-27	
	Non-pigmented F_2	62.50
	Pigmented F_2	32.43
Average for F_2 segregates		
	Non-pigmented	74.17
	Pigmented	41.52

Table 9. Gossypol and tannin content in some *G. arboreum* genotypes.

Sl. No.	Genotype	Gossypol (%)	Tannin (%)
1. G 27		1.58 d	0.74 d
2. Lohit		1.40 c	0.84 d
3. * Pigmented F ₂		1.20 b	0.47 c
4. Cernuum		1.24 b	0.41 bc
5. CJ 73		1.02 a	0.31 b
6. * Nonpigmented F ₂		0.99 a	0.17 a
CD at 5% (t)		0.10	0.12

* Samples from the F₂ segregates of the cross Daulat × Lohit.

(1.20–1.58%) and were also rich in tannin content (0.41–0.84%). The spotted bollworm incidence was found to be negatively correlated with gossypol ($r = -0.7133$) and tannin content ($r = -0.6420$). Gossypol and tannins were also significantly associated between themselves ($r = 0.9040$). Gossypol is the principal antibiotic compound in the cotton plant, and is also genetically inherited (Lee *et al* 1968; Rhyne and Smith 1965; Wilson and Smith, 1976).

The spotted bollworm incidence in *arboreum* varieties seems to be largely influenced by the gossypol and tannin content. On the basis of these results, it is suggested that while selecting plants resistant to spotted bollworms in F₂ populations, the plant pigmentation (red-pigmented) may be used as an important character along with the gossypol and tannin content of the genotypes.

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References

- A.O.A.C. 1975 Official methods of analysis. A.O.A.C., P.O.B. 540, Benjamin Franklin Station, Washington, D.C. 20044. pp. 1094
- Avidov Z and Harpaz J 1969 *Plant pests of Israel* (Jerusalem : Israel University Press)
- Bailey J C, Maxwell F G and Jenkins J N 1967 Mortality of boll weevils in squares of genotypically different lines of cotton ; *J. Econ. Entomol.* 6 1279–1280
- Bottinger G T, Sheehan E T and Lukefahr M J 1964 Relation to gossypol content of cotton plant to insect resistance ; *J. Econ. Entomol.* 57 283–285
- Brazzel J R and Martin D F 1956 Resistance of cotton to pink bollworm damage ; *Bull. Tex. Agric. Exp. Sta.* No. 843, pp. 20

- Brazzel J R and Martin D F 1959 Pink bollworm resistance in cotton ; *J. Econ. Entomol.* **52** 385-390
- Butani D K 1974 Insect pests of cotton. XVII. Effects of cotton varieties, cultural practices and fertilizer on infestation by bollworms ; *Colon et Trop.* **29** 237-240
- Carter C M and Lyman C M 1969 Reactions of gossypol with amino acids and other amino compounds ; *J. Am. Oil Chem. Soc.* **46** 649-653
- Chakravarty S C and Sahni V M 1972 Biochemical basis of resistance to jassids (*Empoasca* spp.) in *G. hirsutum*. Cotton ; *Indian Agric.* **16** 45-48
- Chan B G and Waiss Jr, A C 1978 Condensed tannin, an antibiotic chemical from *Gossypium hirsutum* ; *J. Insect Physiol.* **24** 113-118
- Chang G S, Meng H L and Bao J A 1963 The status of spotted bollworms, *Earias fabia* (Stoll) and *E. insulana* (Boisd.) after the changes of cultural system in Lukang Poashan District, Yunnan Province ; *Acta Entomol. Sin.* **12** 28
- Douglas A G 1966 Selection in cotton for antibiosis to boll weevil ; *J. Econ. Entomol.* **59** 32-34
- Eagle E, Hall C M, Castellan L E and Miller C B 1950 Effect of fractionation and treatment on the acute oral toxicity and gossypol and gossypurin content of cotton seed pigment glands ; *J. Am. Oil Chem. Soc.* **27** 300-303
- Elliger C A, Chan B G and Waiss Jr. A C 1978 Relative toxicity of minor cotton terpenoides compared to gossypol ; *J. Econ. Entomol.* **71** 161-164
- Gillham F E M 1965 Evolutionary significance of glands and their importance in cultivated cotton ; *Emp. Cotton. Grow. Rev.* **112** 101-103
- Gray J R, Marby T J, Bell A A and Stipanovic R D 1976 P-hemigossypolone: a sesquiterpenoid aldehyde quinone from *Gossypium hirsutum* ; *J. Chem. Soc. Chem. Commun.* pp. 109-110
- Hussain M A and Khan M H 1940 Studies on *Platyedra gossypiella* Saunders in Punjab. IX. Relative incidence on exotic and indigenous varieties of cotton ; *Indian J. Entomol.* **2** 45-57
- Lee J A, Cockerham C A and Smith F H 1968 The inheritance of gossypol level in *Gossypium*. I. Additive, dominance, epistatic and maternal effects associated with seed gossypol in two varieties of *Gossypium hirsutum* ; *Genetics* **59** 285-298
- Lukefahr M J and Houghtaling J E 1969 Resistance of cotton strains with high gossypol content to *Heliothis* spp. ; *J. Econ. Entomol.* **62** 588-597
- Lukefahr M J, Houghtaling J E and Cruhm D G 1975 Suppression of *Heliothis* spp. with cottons containing combinations of resistant characters ; *J. Econ. Entomol.* **68** 743-746
- Lukefahr M J, Noble C W and Houghtaling J E 1966 Growth and infestation of bollworms and other insects on glanded and glandless strains of cotton ; *J. Econ. Entomol.* **59** 817-820
- Lukefahr M J, Shaver T N, Cruhm D G and Houghtaling J E 1974 Location, transference and recovery of a *Heliothis* growth inhibition factor present in three *Gossypium hirsutum* race stocks ; *Beltwide Cotton Production Research Conference Proceedings*, 1974
- Lyman C M, Balliga B P and Margaret S W 1959 Reactions of proteins with gossypol ; *Arch. Biochem. Biophys.* **84** 486-497
- Meisner J, Kehat M, Zur M and Asner K R S 1977 The effect of gossypol on the larvae of spiny bollworm, *Earias insulana* ; *Entomol. Exp. Appl.* **22** 301-303
- Meisner J, Ishaaya I, Asher K R S and Arom M Z 1978 Gossypol inhibits protease and amylase activity of *Spodoptera littoralis* larvae ; *Ann. Ent. Soc. Am.* **71** 5-8
- Oliver B F, Maxwell F G and Jenkins J N 1970 A comparison of the damage done by the bollworm to glanded and glandless cottons ; *J. Econ. Entomol.* **63** 1328-1329
- Oliver B F, Maxwell F G and Jenkins J N 1971 Growth of bollworm on glanded and glandless cotton ; *J. Econ. Entomol.* **64** 396-398
- Reed W 1974 Populations and host plant preferences of *Earias* spp. (Lepidoptera : Noctuidae) in East Africa ; *Bull. Entomol. Res.* **64** 33-44
- Rhyné C L and Smith F H 1965 Genetic aspects of gossypol content of leaves and flower buds of *Gossypium* ; *J. Heredity* **56** 242-247
- Shaver T N and Parrot W L 1970 Relationship of larval age to toxicity of gossypol to bollworm, tobacco budworm and pinkbollworms ; *J. Econ. Entomol.* **63** 1802-1804

- Singh H G, Mathur R K and Yadava H M 1965 A study of the thickness of green mature bolls in relation to the incidence of pink bollworm (*Pectinophora gossypiella* Saund.); *Indian Cotton J.* 19 253-255
- Singh M, Agarwal R A and Katiyar K N 1972 Preliminary studies on the inheritance to pink bollworm (*Pectinophora gossypiella* Saunders) resistance in *Gossypium arboreum*; *Entomologist's Newsletter* 2 40-41
- Singh M, Joshi A B and Agarwal R A 1976 Genetics of cotton bollworms (*Pectinophora gossypiella* Saund.) resistance in an intervarietal cross of *Gossypium arboreum* L.; *Coton et Fibres Trop.* 31 369-372
- Sohi G S 1964 Pests of cotton; In: *Entomology in India* (ed.) N C Pant Entomological Society of India pp. 111-148
- Stipanovic R D, Bell A A and Lukefahr M J 1976 Natural insecticides of cotton. Structural analysis and toxicity of heliocides. 172nd Nat. Mfg. Amer. Chem. Soc. 29 Sept. 1976, San Francisco; *Pest. Div. Abs.* No. 78
- Stipanovic R D, Bell A A, O'Brein D H and Lukefahr M J 1977 Heliocide-H₂. An insecticidal sesquiterpenoid from cotton (*Gossypium*); *Tetrahedron Lett.* 567-570
- Tanksley T D, Neumann H, Lyman L M, Pace C M and Prescott J M 1970 Inhibition of pesinogen activation by gossypol; *J. Biol. Chem.* 245 6456-6461
- Walker R L 1952 Spiny bollworm of cotton in Iraq; *FAO Plant Prot. Bull.* 1 42
- Wilson F D and Shaver T N 1973 Glands, gossypol content and tobacco budworm development in seedlings and floral parts of cotton; *Crop Sci.* 13 107-110
- Wilson F D and Smith J N 1976 Some genetic relationships between gland density and gossypol content in *Gossypium hirsutum* L.; *Crop Sci.* 16 830-832
- Yang H C and Davis D D 1976 Variations in gossypol concentrations of flower buds of cotton; *Curr. Sci.* 16 485-488

Some biometric studies of certain closely related species of the genus *Arius* (Pisces : Siluriformes : Ariidae)

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Abstract. The marine catfish genus *Arius* of the family Ariidae comprising 21 species have been divided into six complexes and three groups based on interspecific relationships and morphometric affinities. In this paper the *maculatus* complex of four species, viz., *Arius maculatus*, *Arius arius*, *Arius gagora* and *Arius jella* has been critically examined in respect of a selected list of 20 morphological characters based on examination of a large series of examples collected first hand by the authors. The samples have been statistically analysed, and the range of variation in respect of each character as exhibited by each species has been delineated. The probability significance test has been made to establish the interspecific relationship.

Keywords. Biometric study ; *Arius* species ; Ariidae.

1. Introduction

The genus *Arius* Valenciennes, 1840 forms a commercially important group of marine catfishes comprising 21 species from India, Pakistan, Bangladesh, Burma and Sri Lanka. Most of the species are marine often entering estuarine waters and occasionally even in freshwaters such as *A. acutirostris*, *A. burmanicus* and *A. gagora* etc. About 80% of the total catfish landing in our country is of *Arius* species. Despite the economic value of these fishes, the taxonomic identity of most of the species is in a state of confusion. The main reason for such ambiguity is because earlier workers depended mainly on one or two characters which were highly variable interspecifically if not associated with the changes in growth or sex.

Day (1877, 1889) gave a comprehensive account of 23 species by using the anal fin counts, relative head length and eye diameter as diagnostic characters, besides the shape and size of teeth bands on the palate. Weber and de Beaufort (1913) also utilised the dentition pattern, besides the shape of the occipital process for separating the species of this genus. Smith (1945) considered the dentition pattern as one of the very important taxonomic character and stated, "the most important character for separating the species are teeth." Chandy (1954) framed a key mainly based on the dentition pattern on the palate, for the identification of *Arius* species present in the NZC of ZSI, Calcutta. Subsequent ichthyologists also relied upon this character (Munro, 1955 ; Smith, 1962 ; Wongratana and Bathia, 1974 ; Misra, 1976). Taylor (1978) adopted the length of the median

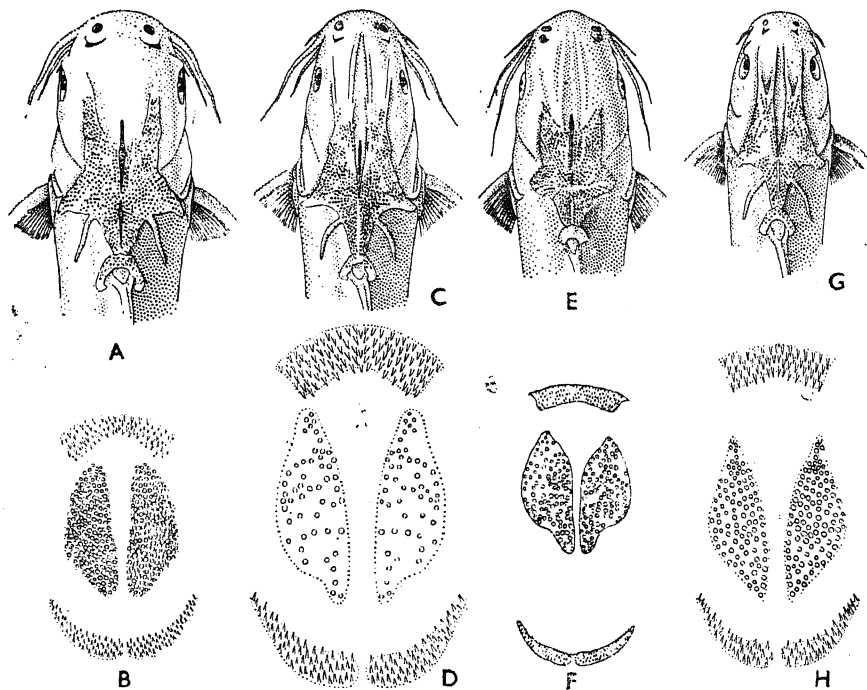
longitudinal groove on the head, the shape of the bony shield for separating *Arius* species of western central Atlantic (Fishing Area 31).

It may be seen that for separating the various species of *Arius* the pattern of teeth patches on the palate still remains to be an unavoidable necessity. However, it may be indicated that whereas the basic contour, the number and position of the patches remain constant, the size, number and nature of the teeth themselves vary highly and alter considerably with age and growth. Earlier ichthyologists seem to be unaware of this fact and established species like *A. serratus* Day, *A. malabaricus* Day, *A. satparanus* Chaudhuri for such variants which are invalid (Jayaram and Dhanze 1978a, 1981).

Based on the number and contour of the patches we have placed the 21 species of *Arius* in six complexes under three groups. The constituent species of each complex are closely interrelated and some may even prove later either to be synonyms or subspecies. In this paper 'maculatus' complex which has four species (*A. maculatus*, *A. arius*, *A. gagora* and *A. jella*) (Text-figures 1A-H), have been analysed to determine their interspecific affinities and systematic status.

2. Materials and methods

The material for this study is based on 430 specimens collected by the authors during extensive survey tours of the entire eastern coast and a part of southwest



Text Figure 1 A.—*A. maculatus* (dorsal view of head). B.—*A. maculatus* (dentition). C.—*A. gagora* (dorsal view of head). D.—*A. gagora* (dentition). E.—*A. arius* (dorsal view of head). F.—*A. arius* (dentition). G.—*A. jella* (dorsal view). H. *A. jella* (dentition).

(Figures A—E, G and H, after Chandy, 1953, Figure F—after Chaudhuri 1916).

coast of India. The specimens present in the National Zoological Collections of the Zoological Survey of India, Calcutta, have also been examined. Fresh material of species such as *A. jella* and *A. maculatus* were collected and studied by the second author (KCF) during the FAO consultation, Cochin in 1980. A total of 45 characters were mensurated and of which 20 alone are selected for the statistical analysis. All the measurements were taken with dial calipers to the nearest half of a millimeter for the size range upto 150 mm and by measuring tape above this size.

Taxonomic characters are generally found to intergrade or overlap between closely related species when a large series of specimens are studied. The reliability or otherwise of such characters are to be evaluated. Different methods of measuring intergradation or divergence have been proposed (Davenport and Blankinship, 1898 ; Pearl, 1930 ; Ginsburg, 1938 ; Simpson and Roe, 1939 ; Amadon, 1949 ; Snedecor, 1956 ; and Simpson *et al* 1960). Methods devised by Simpson *et al* (*op. cit.*) for the comparison of two populations irrespective of their taxonomic identity seems to be useful here. The "Student's *t*-test" to determine the probability value at 95% confidence intervals have been applied. Before deducing any numerical conclusion, a hypothesis was set forth that all the specimens of different populations examined belong to a same species, and the universally used rejection value of 5 per cent was chosen as a criteria for the rejection of this hypothesis. However, the establishment of the significance of a difference between two species by numerical derivation is not in itself a zoological conclusion. Thus the numerical expressions for each character were further compared or rather standardized by employing geometrical expressions proposed by Dice and Leraas (1936), and later on adopted with some modification by Hubbs and Perlmutter (1942), Pillay (1951), Hubbs (1952), and Winterbottom (1980). In this method, for each character the range, mean, one standard deviation and one standard error on each side of the mean were delineated on the graph. The degree of overlap or separation of the standard deviations in respect of the arithmetic mean of each species was determined.

3. Results

Tables 1-6 and graphs 1-20 present the biometric comparison of the four species with each other for all the 20 characters selected.

3.1. *A. maculatus* vs. *A. arius*

It is seen that excepting the head length, in respect of all other characters the two species have a probability of less than 0.1% and are significantly different (table 1). From the graphs XIV-XVI, XVIII, XX, Dice diagram A and B in each, it is seen that the mean of each population as well as standard deviation (S) diverge to a considerable degree, thereby justifying the separate specific status of *A. maculatus* and *A. arius*. Both the species have a single large oval patch of teeth on each side of the palate (text-figure 1 B, F). Further, the two species can be morphologically distinguished by the size and position of the eye. The eye diameter is 18.50% in *A. maculatus* vs. 21.40% in *A. arius* in the head length ; 33.30% vs 45.60% in the interorbital width and 57.35% vs. 63.30% in snout length,

Table 1. Biometrical comparison of various morphometric data for *A. maculatus* vs. *A. arius* from different localities of east and west coast of India.

67 specimens of <i>A. maculatus</i> (Thunberg)			81 specimens of <i>A. arius</i> (Hamilton)				
Range	Mean $\bar{X}_1 \pm s\bar{x}$	S_1	Range	Mean $\bar{X}_2 \pm s\bar{x}$	S_2	t	p
LH/TL %	18.60-25.00	21.1238 \pm .23	18.87-24.32	20.7239 \pm .18	1.3642	1.3796	10-20%
HB/TL %	15.13-21.58	18.3448 \pm .28	13.52-21.50	16.3244 \pm .19	1.4744	6.4097	<0.1%
LH/SL %	23.29-30.12	26.7961 \pm .28	24.65-29.48	26.6695 \pm .18	1.3996	0.4185	<60.70%
HB/SL %	18.75-26.97	22.9738 \pm .28	17.77-26.23	21.0027 \pm .21	1.6385	5.7282	<0.1%
PDL/SL %	32.41-39.44	36.3168 \pm .23	35.25-40.45	37.0227 \pm .20	1.1529	-2.6251	1.00%
PAL/SL %	66.67-75.00	69.6174 \pm .31	63.93-73.08	69.8702 \pm .29	2.278	-0.6025	<0.1%
PPL/SL %	21.72-30.98	25.9972 \pm .36	22.40-27.41	24.7078 \pm .18	1.3965	-3.2817	0.1%
WDF/WAF %	50.00-72.22	59.7017 \pm .55	52.59-69.70	61.6339 \pm .51	3.9983	-2.5670	<1.00%
WH/LH %	63.16-84.09	72.8778 \pm .64	62.60-78.57	70.1412 \pm .45	3.51167	3.5376	<0.1%
LS/LH %	55.26-71.59	65.4108 \pm .57	52.00-70.00	60.6015 \pm 1.26	4.7358	5.4483	<0.1%
ED/LH %	28.00-37.25	32.3725 \pm .29	16.00-26.67	33.8687 \pm .21	1.6373	-4.2560	<0.1%
INW/LH %	14.71-22.22	18.4687 \pm .21	15.29-21.77	18.2822 \pm .18	2.2268	-7.9926	<0.1%
IOW/LH %	44.19-67.39	56.1914 \pm .67	41.07-52.75	47.0943 \pm .43	1.4063	11.0167	<0.1%
ED/LS %	39.47-66.67	57.3725 \pm .82	43.24-77.78	63.3336 \pm 1.07	2.7251	12.8595	<0.1%
INW/LS %	54.55-76.67	65.9427 \pm .80	44.64-61.90	54.0478 \pm .55	8.3560	-4.3693	<0.1%
INW/WS %	42.86-57.50	49.0378 \pm .45	40.91-51.22	46.2846 \pm .35	4.3287	12.3950	<0.1%
ED/IOW %	25.42-47.06	33.2938 \pm .56	32.65-60.87	45.6090 \pm .86	2.7336	-4.8603	<0.1%
WPMT/LPMT %	15.00-22.22	19.0363 \pm .28	16.67-30.00	23.7321 \pm .38	6.7380	11.7740	<0.1%
HCPD/LCPD %	47.62-63.41	53.8982 \pm .62	38.54-59.65	49.5717 \pm .61	2.9768	9.8383	<0.1%
					4.8020	-4.9852	<0.1%

ED = Eye diameter; HB = height of body; HCPD = height of caudal peduncle; HH = height of head; INW = inter-nostril width; IOW = inter-orbital width; LCPD = length of caudal peduncle; LH = length of head; LPMT = length of premaxillary band of teeth; LS = length of snout; P = observed probabilities for confidence intervals given in the Simpson *et al* (1960), Appendix Tab. II, for the corresponding calculated χ^2 value; PAL = pre-anal length; PDL = pre-dorsal length; PPL = pre-pectoral length; S_1, S_2 = standard deviation of first and second population respectively; SL = standard length; $s\bar{x}$ = standard error; TL = total length; WAF = width of anal fin base; WDF = width of dorsal fin base; WH = width of head; WPMT = width of pre-maxillary band of teeth; \bar{X}_1, \bar{X}_2 = arithmetic mean of the first and second population respectively.

3.2. *A. maculatus* vs. *A. gagora*

The two species differ significantly in respect of 14 characters having the probability of less than 5% (table 2) and the standard deviation not overlapping with arithmetic mean of the other in respect of 11 characters (Graphs I, II, V, XI-XIII, XV, XVI, XVIII-XX, Dice diagram A and D in each).

A. gagora and *A. maculatus* have a single oval large patch of teeth on each side of the palate (Text-figure 1 B, D), though the teeth may be set sparsely in the former and densely packed in the latter. However, we have observed sparse arrangement of teeth in a few adult male specimens of *A. maculatus* also. Further, *A. gagora* is significantly different from *A. maculatus* in respect of eye size and internostril distance. The eye diameter in snout length is 57.37% in *A. maculatus* vs. 39.03 in *A. gagora*; the internostril width in snout length is 65.94% vs. 48.17%. It may be mentioned here that in respect of the other so called significant characters such as predorsal length, width of dorsal fin, height of head etc., the difference is not very high. From the distributional pattern of both the species it would seem that *A. maculatus* is replaced by *A. gagora* in the Hooghly estuarine system.

3.3. *A. maculatus* vs. *A. jella*

From the data presented in table 2, it can be seen that only in respect of 10 characters the probability is less than 5% and in respect of the remaining ten characters it is more than 5%. Among the significant characters, the least depth of caudal peduncle in its length is 53.89% in *A. maculatus* vs. 43.96% in *A. jella*; internostril width in snout length 65.94% vs. 60.42% and the size and position of eyes are noteworthy. Further, *A. jella* is clearly separable from *A. maculatus* by the length of the pectoral spine which in *A. jella* is longer than the dorsal spine. In most species of *Arius* the pectoral spine is equal or shorter than the dorsal spine. We have examined specimens of all sizes in both the sexes in each species and have not found any variation in respect of this character (Graphs XII, XVIII-XX, Dice diagram A and C in each).

3.4. *A. arius* vs. *A. jella*

These two species differ statistically in respect of ten characters in the fact that their probability is less than 5%. Table 4 and Graphs XII-XIV, Dice diagram B and C in each, indicate the degree of intergradation or divergence. Here again the size and position of the eye appears to be an important character. The eye in head length is 21.30% in *A. arius* vs. 15.95% in *A. jella*; in snout length 63.30% vs. 50.35%; in interorbital width 45.60% vs. 33.90%. Further, as stated already *A. jella* is separable by the character of pectoral spine being longer than dorsal spine as compared to other species of *Arius*. *A. jella* is darker in colour than *A. arius*.

3.5. *A. arius* vs. *A. gagora*

The systematic position of these two species is slightly vague. *A. gagora* is not very well represented and apparently not collected extensively as *A. arius*. One of us (JRD) was able to obtain four fresh specimens (195-245 mm SL) of *A. gagora*

Table 2. Biometrical comparison of various morphometric data for *A. maculatus* from different localities of east and west coast of India vs. *A. gagora* from Hooghly Estuary,

	67 specimens of <i>A. maculatus</i> (Thunberg)				18 specimens of <i>A. gagora</i> (Hamilton)			
	Range	Mean $\bar{x}_1 \pm s\bar{x}$	S_1	Range	Mean $\bar{x}_2 \pm s\bar{x}$	S_2	t	p
LH/TL %	18.60-25.00	21.1238 \pm .23	1.7656	22.41-25.38	23.7500 \pm .87	1.5070	-2.5120	<0.01%
HB/TL %	15.13-21.58	18.5300 \pm .34	2.3920	17.24-18.28	17.6100 \pm .33	0.5812	0.6050	<0.01%
LH/SL %	23.29-30.12	26.7960 \pm .28	2.0935	27.66-29.28	28.8740 \pm .52	0.7168	-2.1783	<0.01%
HB/SL %	18.75-26.97	22.9738 \pm .28	2.0858	19.23-22.55	20.8680 \pm .60	1.3336	2.1883	<0.01%
PDL/SL %	32.41-39.44	36.3168 \pm .23	1.7280	37.45-42.07	39.4160 \pm .77	1.7134	-3.7971	<0.01%
PAL/SL %	66.67-75.00	69.6174 \pm .31	2.3284	70.61-74.52	71.9540 \pm .73	1.6371	-2.1674	<0.01%
PPL/SL %	21.72-30.98	25.9972 \pm .36	2.7055	25.53-28.85	26.5642 \pm .59	1.3234	-0.4575	<0.01%
WDF/WAF %	50.00-72.22	59.7017 \pm .55	4.1754	57.89-71.43	65.5316 \pm 2.51	5.6225	-2.8623	<0.01%
WH/LH %	63.16-84.09	72.8778 \pm .64	4.8220	65.15-76.92	71.3142 \pm 1.95	4.3687	0.6910	<0.01%
HH/LH %	55.26-71.59	65.4108 \pm .57	4.2906	53.66-67.69	59.2222 \pm 2.61	5.8275	2.9534	<0.01%
LS/LH %	28.00-37.25	32.3725 \pm .29	2.1601	37.40-41.54	38.6443 \pm .74	1.6519	-6.2475	<0.01%
ED/LH %	14.71-22.22	18.4687 \pm .21	1.5800	13.64-16.18	14.9624 \pm .52	1.1614	4.7839	<0.01%
INW/LH %	17.91-23.66	21.2597 \pm .20	1.5241	17.65-19.70	18.5842 \pm .33	0.7375	3.8333	<0.01%
IOW/LH %	44.19-67.39	56.1914 \pm .67	4.5746	42.11-45.45	43.7321 \pm .56	1.2436	5.9883	<0.01%
ED/LS %	39.47-66.67	57.3725 \pm .82	6.2239	36.00-42.86	39.0343 \pm 1.46	3.2731	6.4222	<0.01%
INW/LS %	54.55-76.67	65.9427 \pm .80	5.0087	44.44-52.00	48.1741 \pm 1.34	3.0009	6.4539	<0.01%
INW/WS %	42.86-57.50	49.0378 \pm .45	3.4019	46.15-56.52	50.5662 \pm 1.99	4.4440	-0.9247	<0.01%
ED/IOW %	25.42-47.06	33.2938 \pm .56	4.2541	30.00-37.50	34.2721 \pm 1.49	3.3272	-0.4942	<0.01%
WPMT/LPMT %	15.00-22.22	19.0363 \pm .28	2.1150	23.33-31.25	25.9160 \pm 1.37	3.0682	-6.5967	<0.01%
HCPD/LCPD %	47.62-63.41	53.8982 \pm .62	4.6490	40.00-46.75	42.9453 \pm 1.38	3.0915	5.0996	<0.01%

For abbreviations see table 1.

Table 3. Biometrical comparison of various morphometric data for *A. maculatus* from different localities of east and west coast vs. *A. jella* only from east coast of India.

	67 specimens of <i>A. maculatus</i> (Thunberg)				12 specimens of <i>A. jella</i> (Day)			
	Range	Mean $\bar{x}_1 \pm s\bar{x}$	S_1	Range	Mean $\bar{x}_2 \pm s\bar{x}$	S_2	t	p
LH/TL %	18.60-25.00	21.1238 \pm .23	1.7656	19.82-22.13	21.0780 \pm .36	0.8903	0.1433	80-90 %
HB/SL %	15.13-21.58	18.5300 \pm .34	2.5920	16.46-19.64	18.3533 \pm .46	1.1381	0.1638	5 %
LH/SL %	23.29-30.12	26.7960 \pm .28	2.0935	24.67-27.94	26.0325 \pm .45	1.2626	0.9963	30-40 %
HB/SL %	18.75-26.97	22.9738 \pm .28	2.0858	17.20-24.44	21.3337 \pm .77	2.1917	2.0419	5 %
PDL/SL %	32.41-39.44	36.3168 \pm .23	1.7280	35.56-38.71	36.8312 \pm .39	1.1093	-0.8104	More than 90 %
PAL/SL %	66.67-75.00	69.6174 \pm .31	2.3284	65.71-72.07	69.0900 \pm .81	2.2873	0.5936	60 %
PPI/SL %	21.72-30.98	25.9972 \pm .36	2.7055	22.38-24.71	23.6812 \pm .29	0.8171	2.3858	2 %
WDF/WAF %	50.00-72.22	59.7017 \pm .55	4.1754	62.50-69.23	65.5857 \pm 1.00	2.6487	-3.8391	Less than 0.1 %
WH/LH %	63.16-84.09	72.8778 \pm .64	4.8220	68.27-78.38	72.5950 \pm 1.30	3.6856	0.1577	90 %
HH/LH %	55.26-71.59	65.4108 \pm .57	4.2906	50.98-66.67	58.3900 \pm 1.82	5.1389	4.2014	Less than 0.1 %
LS/LH %	28.00-37.25	32.3725 \pm .29	2.1601	27.45-34.62	31.9212 \pm .98	2.7583	0.5248	60 %
ED/LH %	14.71-22.22	18.4687 \pm .21	1.5800	15.38-16.84	15.9662 \pm .20	0.5620	4.4029	Less than 0.1 %
INW/LH %	17.91-23.66	21.2597 \pm .20	1.5241	15.69-23.16	19.2425 \pm .73	2.0512	3.2888	0.1 %
IOW/LH %	44.19-67.39	56.1914 \pm .67	4.5746	41.18-50.00	47.3887 \pm 1.33	3.7722	5.1410	Less than 0.1 %
ED/LS %	39.47-66.67	57.3725 \pm .82	6.2239	44.44-57.14	50.3725 \pm 1.67	4.7330	3.0281	0.1 %
INW/LS %	54.55-76.67	65.9427 \pm .80	6.0087	55.56-70.97	60.4250 \pm 1.98	5.5894	2.4226	2 %
INW/WS %	42.86-57.50	49.0378 \pm .45	3.4019	44.44-57.89	51.3862 \pm 1.46	4.1209	-1.7516	10 %
ED/IOW %	25.42-47.06	33.2938 \pm .56	4.2541	30.77-40.00	33.9312 \pm 1.26	3.5598	-0.3997	70 %
WPMT/LPMT %	15.00-22.22	19.0363 \pm .28	2.1150	18.18-31.82	25.3887 \pm 1.79	5.0591	-6.1630	Less than 0.1 %
HCPD/LCPD %	47.62-63.41	53.8982 \pm .62	4.6490	37.04-48.33	43.9687 \pm 1.40	3.7722	5.7148	0.1 %

For abbreviations see table 1

from the river Hooghly at Serampore (W.B.) and eight specimens (120–185 mm SL) from Haldi estuary at Halida 1art (W.B.). From a critical examination it is seen that *A. gagora* is clearly separable from *A. arius* by its shallow median longitudinal groove which extends up to the supraoccipital crest as compared to *A. arius* which has the median longitudinal groove narrow extending only up to the frontal bones (text-figure 1 C, E). Besides, both the species differ in respect of the size of the eye which is larger in *A. arius* than in *A. gagora*; the eye diameter in head length is 21.35% in *A. arius* vs. 14.95% in *A. gagora*; in snout length 63.30% vs. 39.00%; in interorbital width 45.60% vs. 34.25%. Further, 13 characters having less than 5% probability indicate the statistical differences between the two species (Table 5; graphs, I, II, V, XI–XIV, Dice diagram B and D in each.)

3.6. *A. gagora* vs. *A. jella*

Table 6 presents the comparative data in respect of *A. gagora* and *A. jella*. Nine characters showing the probability of less than 5% are delineated in the Dice diagram C and D (graphs I, II, V, VII, XI, XIII, XV). Morphologically the two species can be distinguished by the size and position of the eye and also the relative distance between the pairs of nostrils on each side. The nostrils in *A. jella* are closer to each other on either side than in *A. gagora*. The eye diameter in snout length is 39.00% in *A. gagora* vs. 44.45% in *A. jella*; the internostril width in length of snout 48.20% vs. 55.60%.

4. Discussion

The above analyses of four species forming the *maculatus* complex of the genus *Arius* indicate clearly their close inter-relationship. Morphologically also these species resemble each other in one or other character and in juvenile stages they are hard to separate, more particularly since all of them have a single oval patch of teeth on the palate (text-figure 1 B, D, F, H). The 20 characters which appeared helpful to differentiate these species were utilized for statistical interpretation and the extent of range of variation of each character was computed. The probability significance in respect of each such morphometric character as shown by each species is summarised in table 7.

Of the 20 characters selected there is not a single character which can distinguish each species from the other. The size and position of the eye is most significant followed by the internostril width, snout length and the least depth of the caudal peduncle. The body depth, head width, head length etc., the conventional characters used by the earlier ichthyologists do not appear to be of much help, at least in respect of these four species. Considering the fact that the *maculatus* complex of species are inhabitants of clear oceanic and estuarine waters feeding on carnivorous diet in midwater, the differences in structure and position of the eye seems justified.

Of the four species it is seen that *A. maculatus* and *A. arius* are well established separate populations, each occupying its own separate habitat. Thus *A. maculatus* is extensively distributed in the Arabian sea with stray individuals occasionally caught in Bay of Bengal. *A. arius* on the other hand is extensively found in Bay of Bengal having not been so far recorded south of Portonovo. Moreover,

Table 5. Biometrical comparison of various morphometric data for *A. arius* from different localities of east and west coast of India vs. *A. gagora* only from Hooghly estuary.

81 specimens of <i>A. arius</i> (Day)					18 specimens of <i>A. gagora</i> (Hamilton)				
Range	Mean $\bar{X}_1 \pm \bar{\sigma}_x$	S_1	Range	Mean $\bar{X}_2 \pm \bar{\sigma}_x$	S_2	t	p		
LH/TL %	18.87-24.32	20.7239 \pm .18	1.3642	22.41-25.38	23.7500 \pm .87	1.5070	-3.8275	Less than 0.1%	
HB/TL %	13.52-21.50	16.3243 \pm .19	1.4744	17.24-18.28	17.6100 \pm .33	0.5812	-1.4950	10-20%	
LH/SL %	24.65-29.48	26.6595 \pm .18	1.3996	27.66-29.57	28.8740 \pm .32	0.7158	-3.4826	0.1%	
HB/SL %	17.77-26.23	21.0022 \pm .21	1.6385	19.23-22.55	20.8680 \pm .60	1.3336	0.1779	80-90%	
PDL/SL %	35.25-40.45	37.0227 \pm .20	1.1529	37.45-42.07	39.4160 \pm .77	-1.7134	-2.5898	1-2%	
PAL/SL %	63.93-73.08	69.8702 \pm .29	2.2278	70.61-74.52	71.9540 \pm .73	1.6371	-2.0402	5%	
PPL/SL %	22.40-27.41	24.7078 \pm .18	1.3965	25.53-28.83	26.5642 \pm .59	1.3234	-2.8667	1%	
WDF/WAF %	52.59-69.70	61.6339 \pm .51	3.9983	57.89-71.43	65.5316 \pm 2.51	5.6225	-2.0343	5%	
WH/LH %	62.60-78.57	70.1412 \pm .45	3.5167	65.15-76.92	71.3142 \pm 1.95	4.3687	-0.7051	50%	
HH/LH %	52.00-70.00	60.6015 \pm 1.26	4.7358	53.66-67.69	59.2222 \pm 2.61	5.8275	0.6162	50-60%	
LS/LH %	30.00-37.00	33.8687 \pm .21	1.6373	37.40-41.54	38.6443 \pm .74	1.6519	-6.2666	Less than 0.1%	
ED/LH %	16.00-26.67	21.3634 \pm .29	2.2668	13.64-16.18	14.9624 \pm .52	1.1614	6.2152	0.1%	
INW/LH %	15.29-21.77	18.2822 \pm .18	1.4063	17.65-19.70	18.5842 \pm .33	0.7375	-0.4724	60-70%	
IOW/LH %	41.07-52.75	47.0943 \pm .43	2.7251	42.11-45.45	43.7321 \pm .56	1.2436	2.7204	1%	
ED/LS %	43.24-77.78	63.3336 \pm 1.07	8.3560	36.00-42.86	39.0343 \pm 1.46	3.2731	6.4235	Less than 0.1%	
INW/LS %	44.64-61.90	54.0478 \pm .55	4.3287	44.44-52.00	48.1741 \pm 1.34	3.0009	2.9655	0.1-1%	
INW/WS %	40.91-51.22	46.2846 \pm .35	2.7336	46.15-56.52	50.5662 \pm 1.99	4.4440	-3.2064	0.1%	
ED/IOW %	32.65-60.87	45.6090 \pm .86	6.7380	30.00-37.50	34.2721 \pm 1.49	3.3272	3.5588	Less than 0.1%	
WPM/LPMT %	16.67-30.00	23.7322 \pm .38	2.9768	23.33-31.25	25.9160 \pm 1.37	3.0682	-1.5739	10%	
HCPD/LCPD %	38.64-59.65	49.5717 \pm .61	4.8020	40.00-46.75	42.9453 \pm 1.38	3.0915	3.0222	0.1-1%	

For abbreviations see table I

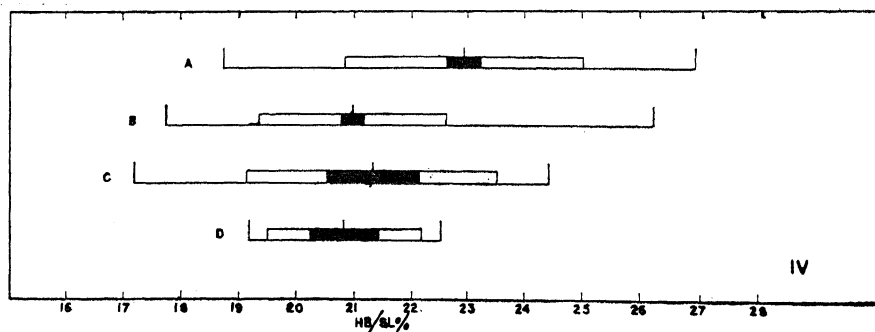
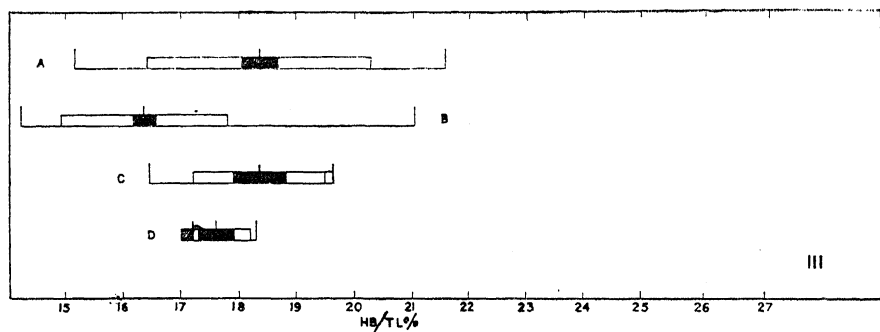
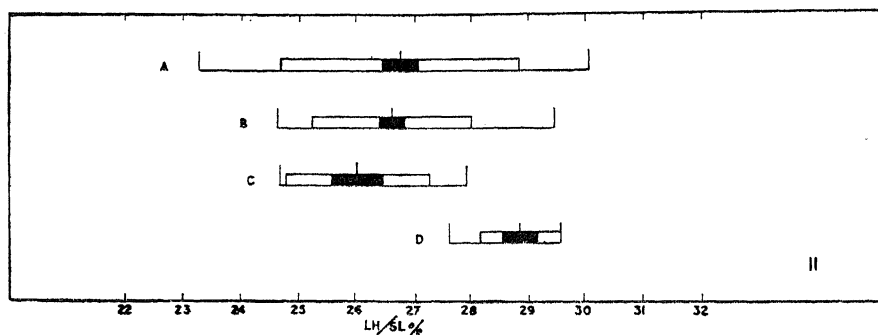
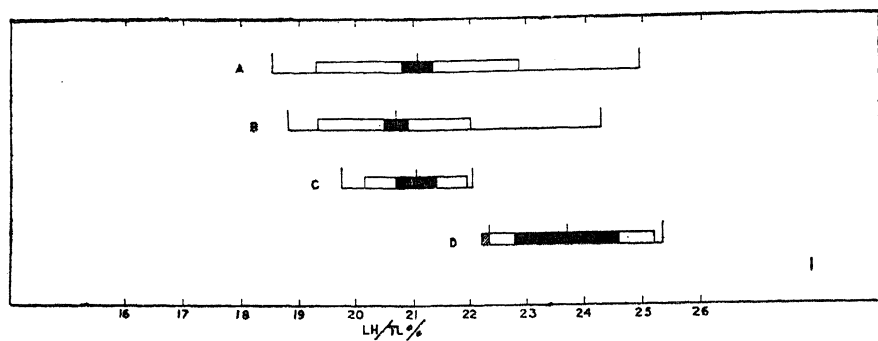
Table 6. Biometrical comparison of various morphometric data for *A. gagora* from Hooghly estuary vs. *A. jella* from Orissa coast.

18 specimens of <i>A. gagora</i> (Hamilton)				12 specimens of <i>A. jella</i> Day			
Range	Mean $\bar{X}_1 \pm s\bar{x}$	S_1	Range	Mean $\bar{X}_2 \pm s\bar{x}$	S_2	t	p
LH/TL %	22.41-25.38	23.7500 \pm .87	1.5070	19.82-22.13	21.0780 \pm .36	0.8903	3.2787 1%
HB/TL %	17.24-18.28	17.6100 \pm .33	0.5812	16.46-19.64	18.3533 \pm .46	1.1381	1.0399 30%
LH/SL %	27.66-29.57	28.8740 \pm .32	0.7158	24.67-27.94	26.0325 \pm .45	1.2626	4.5485 Less than 0.1%
HB/SL %	19.23-22.55	20.8680 \pm .60	1.3336	17.20-24.44	21.3337 \pm .77	2.1917	0.4244 60-70%
PDL/SL %	37.45-42.07	39.4160 \pm .77	1.7134	35.56-38.71	36.8312 \pm .39	1.1093	2.4500 2-5%
PAL/SL %	17.61-74.52	71.9540 \pm .73	1.6371	65.71-72.07	69.0900 \pm .81	2.2873	2.4215 2-5%
PPL/SL %	25.53-28.85	26.5642 \pm .59	1.3234	22.38-24.71	23.6812 \pm .29	0.8171	4.8618 Less than 0.1%
WDF/WAF %	57.89-71.43	65.5316 \pm 2.51	5.6225	62.50-69.23	65.5857 \pm 1.00	2.6487	-0.0237 More than 90%
WH/LH %	65.15-67.92	71.3142 \pm 1.95	4.3687	68.27-78.38	72.5950 \pm 1.30	3.6856	-0.5691 60%
HH/LH %	53.66-67.69	59.2222 \pm 2.61	5.8275	50.98-66.67	58.3900 \pm 1.82	5.1389	0.2703 80%
LS/LH %	37.40-41.54	38.6443 \pm .74	1.6519	27.45-34.62	31.9212 \pm .98	2.7583	4.8825 Less than 0.1%
ED/LH %	13.64-16.18	14.9624 \pm .52	1.1614	15.38-16.84	15.9662 \pm .20	0.5620	-2.1174 5%
INW/LH %	17.65-19.70	18.5842 \pm .33	0.1375	15.69-23.16	19.2425 \pm .73	2.0512	-0.6825 50%
LOW/LH %	47.11-45.45	43.7321 \pm .56	1.2436	41.18-50.00	47.3887 \pm 1.33	3.7722	-2.0682 5-10%
ED/LS %	36.00-42.86	39.0343 \pm 1.46	3.2731	44.44-57.14	50.3725 \pm 1.67	4.7330	-4.6820 Less than 0.1%
INW/LS %	44.44-52.00	48.1741 \pm 1.34	3.0009	55.56-70.97	60.4250 \pm 1.98	5.5894	-4.4657 0.1%
INW/WS %	46.15-56.52	50.5662 \pm 1.99	4.4440	44.44-57.89	51.3862 \pm 1.46	4.1209	-0.3391 70%
ED/OW %	30.00-37.50	34.2721 \pm 1.49	3.3272	30.77-40.00	33.9312 \pm 1.26	3.5598	-0.1719 80-90%
WPMT/LPMT %	23.33-31.25	25.9160 \pm 1.37	3.0682	18.18-31.82	25.3887 \pm 1.79	5.0591	0.2083 80%
HCPD/LCPD %	40.00-46.75	42.9453 \pm 1.38	3.0915	37.04-48.33	43.9687 \pm 1.40	3.7722	-0.4069 60-70%

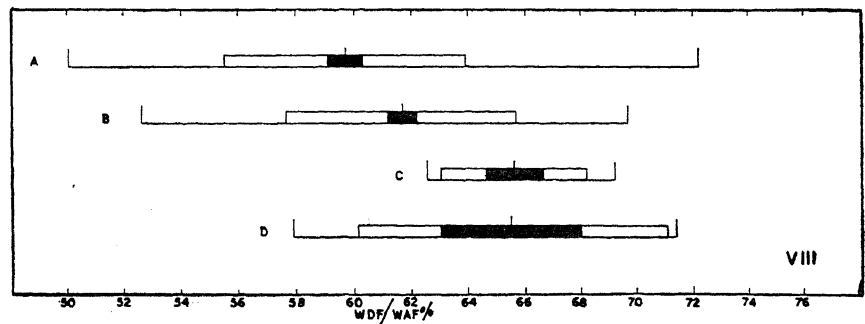
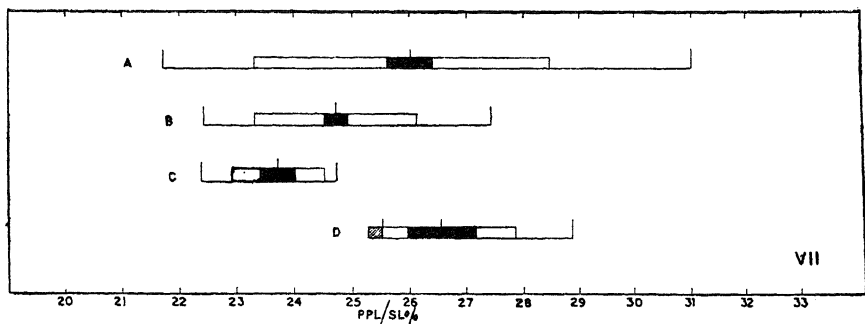
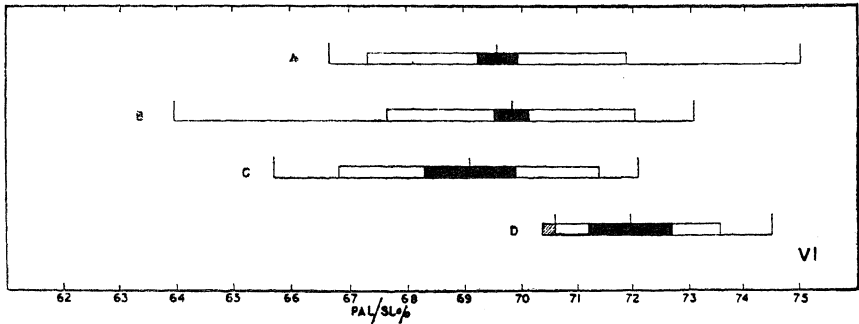
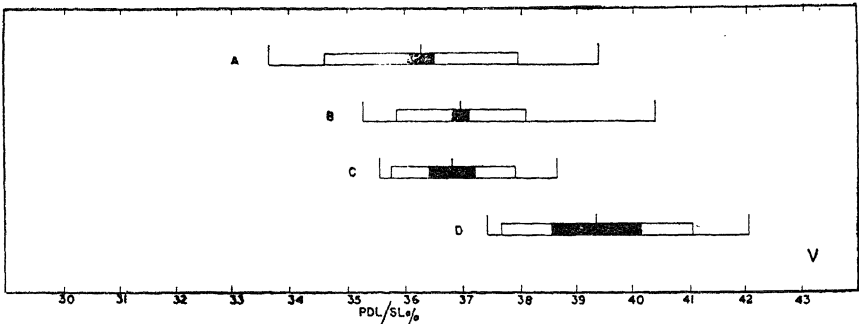
For abbreviations see table I

Table 7. Probability distribution of various morphometric character in respect of six combinations of the four species; *A. maculatus*, *A. arius*, *A. gagora* and *A. jella*.

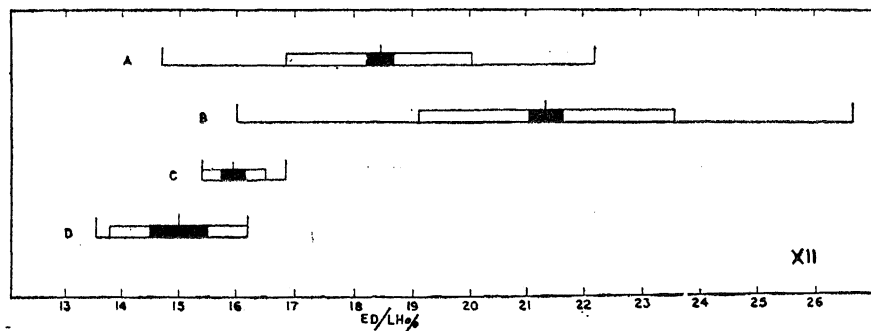
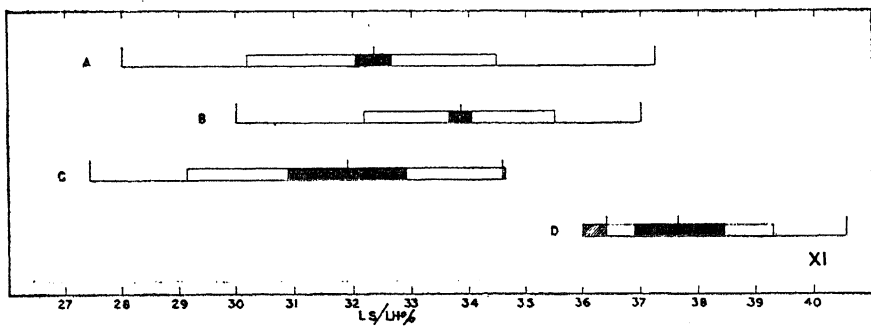
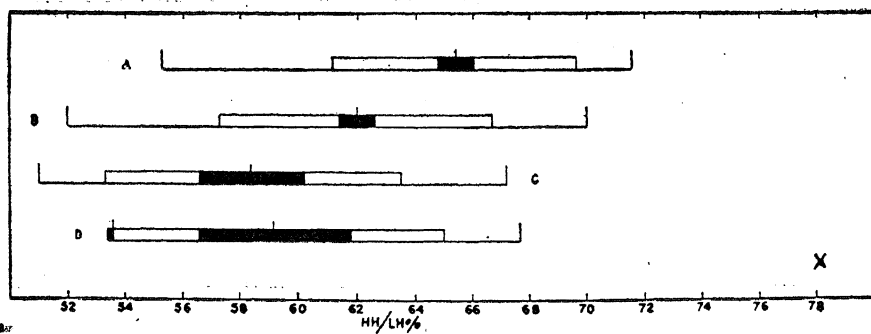
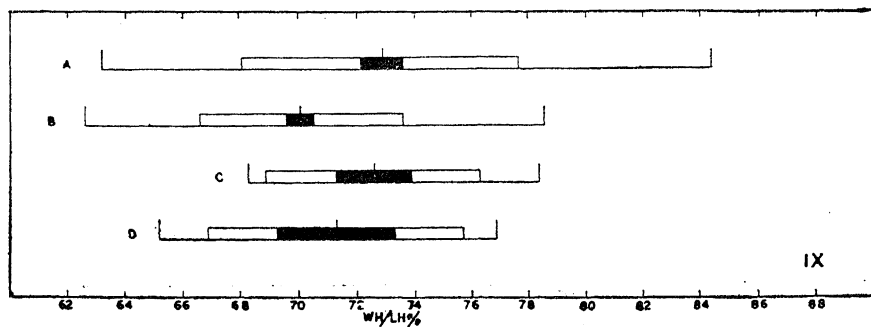
	<i>A. maculatus</i> vs. <i>A. arius</i> (%)	<i>A. maculatus</i> vs. <i>A. gagora</i> (%)	<i>A. maculatus</i> vs. <i>A. jella</i> (%)	<i>A. arius</i> vs. <i>A. gagora</i> (%)	<i>A. arius</i> vs. <i>A. jella</i> (%)	<i>A. gagora</i> vs. <i>A. jella</i> (%)
LH/TL %	10-20	20	80-90	< 0.1	50	1.00
HB/TL %	< 0.1	50-60	50	10-20	0.1	30
LH/SL %	60-70	5	30-40	0.1	20	< 0.1
HB/SL %	< 0.1	5	5	80-90	60	60-70
PDL/SL %	1	< 0.1	More than 90	1-2	60-70	2-5
PAL/SL %	< 0.1	5	60	5	30-40	2-5
PPL/SL %	1	60-70	2	1	5	< 0.1
WDF/WAF %	1	1	< 0.1	5	1	More than 90
WH/LH %	< 0.1	50	90	50	5-10	60
HH/LH %	< 0.1	1	< 0.1	50-60	20	80
LS/LH %	< 0.1	< 0.1	60	< 0.1	0.1-2	< 0.1
ED/LH %	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	5
INW/LH %	< 0.1	< 0.1	0.1	60-70	10	50
IOW/LH %	< 0.1	< 0.1	< 0.1	1	30	5-10
ED/LS %	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1
INW/LS %	< 0.1	< 0.1	2	0.1-1	< 0.1	< 0.1
INW/WS %	< 0.1	30	10	0.1	< 0.1	70
ED/IOW %	< 0.1	60-70	70	< 0.1	< 0.1	80-90
WPM/LPMT %	< 0.1	< 0.1	< 0.1	10	20	80
HCPD/LCPD %	< 0.1	< 0.1	< 0.1	0.1-1	0.1-1	60-70



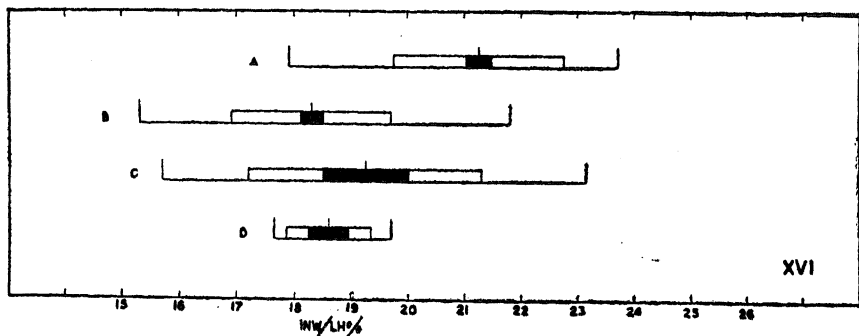
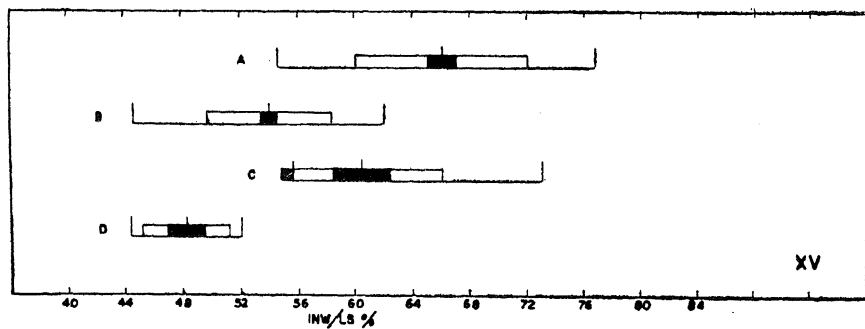
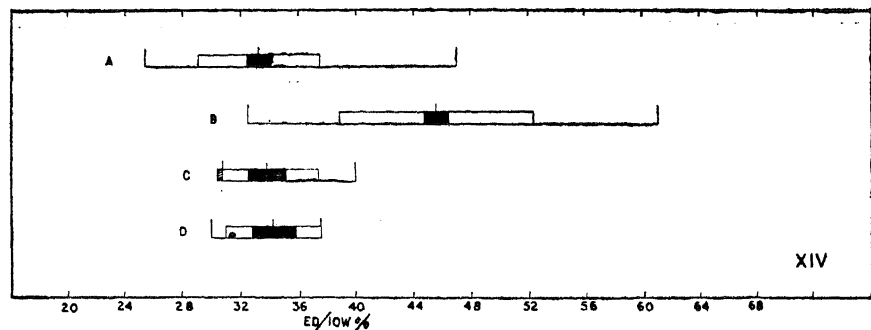
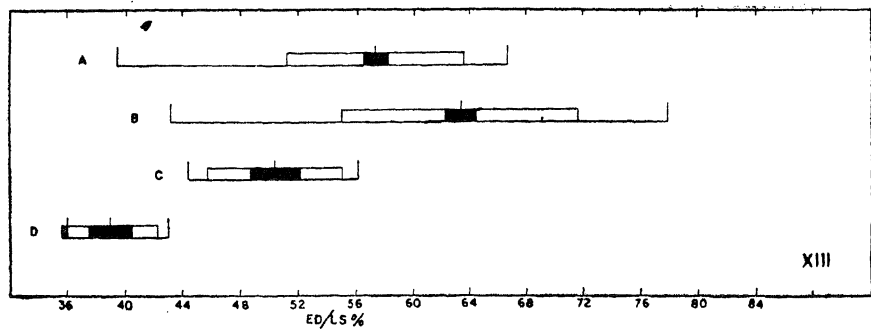
Graphs I-IV



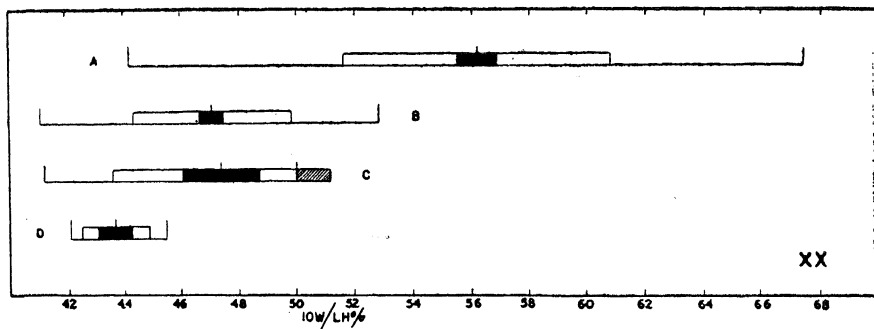
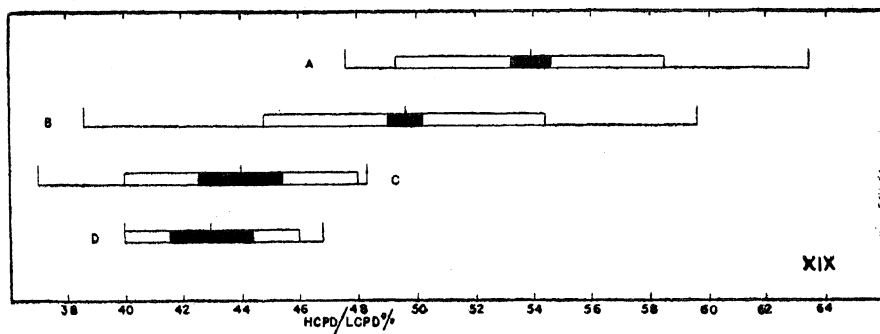
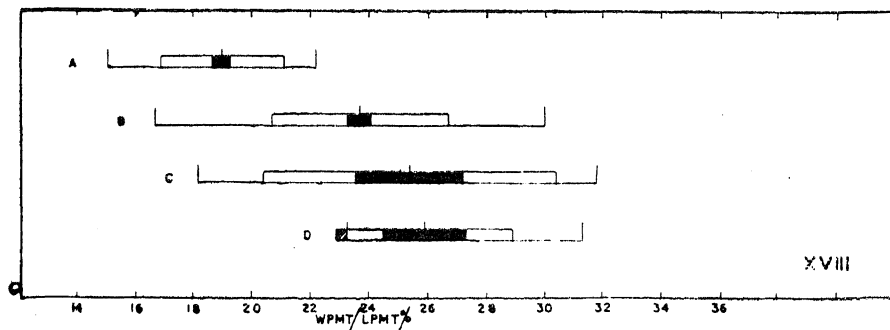
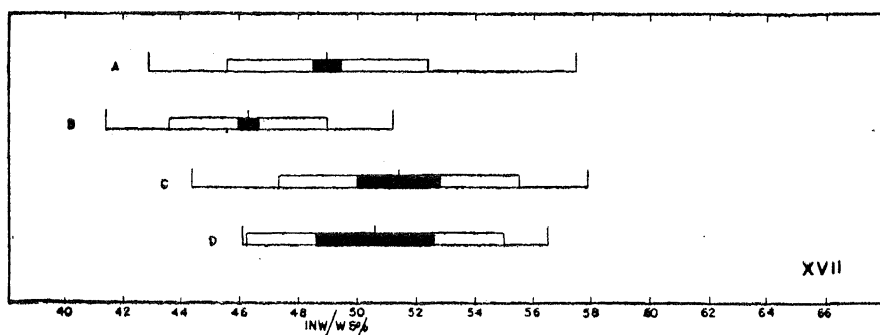
Graphs V-VIII



Graphs IX-XII



Graphs XIII-XVI



Graphs XVII-XX

Graphs I-XX (See for Captions in p. 98)

Table 8. Showing the extent of intergradation in respect of five morphometric characters in *A. gagora* vs. *A. jella*.

Species	Length of head as percentage of total length								Intergradation	
	19·00-19·95	20·00-20·95	21·00-21·95	22·00-22·95	23·00-23·95	24·00-24·95	25·00-25·95			
<i>A. gagora</i>	33·33	33·33	..	33·33	24·995% ≡ Subspecific status		
<i>A. jella</i>	16·66	33·33	33·33	16·66						
Species	Length of head as percentage of standard length								Intergradation	
	24·00-24·95	25·00-25·95	26·00-26·95	27·00-27·95	28·00-28·95	29·00-29·95	= Subspecific status			
<i>A. gagora</i>	20·00	..	80·00	22·50			
<i>A. jella</i>	37·50	12·50	25·00	25·00				
Species	Pre-dorsal length as percentage of standard length								Intergradation	
	35·00-35·95	36·00-36·95	37·00-37·95	38·00-38·95	39·00-39·95	40·00-40·95	41·00-41·95	42·00-42·95		
<i>A. gagora</i>	20·00	20·00	40·00	20·00 16·25% ≡ Subspecific status		
<i>A. jella</i>	25·00	37·00	25·00	12·50			
Species	Pre-anal length as percentage of standard length								Intergradation	
	65·00-65·95	66·00-66·95	67·00-67·95	68·00-68·95	69·00-69·95	70·00-70·95	71·00-71·95	72·00-72·95		73·00-73·95
<i>A. gagora</i>	40·00	20·00	20·00	..	20·00 25·00% ≡ subspecific status
<i>A. jella</i>	12·50	12·50	12·50	12·50	..	25·00	12·50	12·50
Species	Eye diameter as percentage of length of head								Intergradation	
	13·00-13·95	14·00-14·95	15·00-15·95	16·00-16·95						
<i>A. gagora</i>	40·00	..	40·00	20·00						
<i>A. jella</i>	50·00	50·00					30·00%	

A. arius is an inhabitant of brackish water lakes such as Chilka, while *A. maculatus* seems to prefer deeper waters of the open seas. It would seem that *A. maculatus* is replaced by *A. arius* in the Bay of Bengal north of Coromandel coast.

A. gagora is found in the Hooghly estuarine system and is known from comparatively lesser saline waters than the other species of this complex. It is most closely related to *A. jella* which is also known from Orissa and Bengal coast. It is clearly separable from *A. maculatus* and *A. arius* by the relative extension of the median longitudinal groove, besides size and position of the eye (*vide supra*).

The data of probability distribution depicted in table 7 substantiated by Ginsburg's method of analysis (table 8) would seem to indicate that *A. jella* is only a subspecies of *A. gagora*. Pending further studies with the fond hope of obtaining more material of these two species, we have kept *A. jella* as a separate species for the present. It is concluded as such that *A. maculatus* complex comprises of four species : *A. maculatus* (Thunberg, 1792), *A. arius* (Hamilton, 1822), *A. gagora* (Hamilton, 1822) and *A. jella* Day, 1877.

Acknowledgement

We are thankful to the Director, Zoological Survey of India, Calcutta, for facilities.

References

- Amadon D 1949 The seventy five per cent rule for subspecies ; *Condor* 51 250-258
- Chandy M 1954 A key for the identification of the catfishes of the genus *Tachysurus* Lacépède with a catalogue of the specimens in the collection of the Indian Museum (Zool. Surv.); *Rec. Indian Mus.* 51 (1) 1-18, 3 pls. text figures.
- Devenport C B and Blankinship J W 1898 A precise criterion of species ; *Science* (N.S.) 7 684-695
- Day F 1877 *The fishes of India* ; London, Wm. Dawson and Sons 778 pp. 198 pl.
- Day F 1889 *The fauna of British India including Ceylon and Buma*, Fishes 1 169-192, Taylor and Francis, London
- Dhanze J R and Jayaram K C 1979 The family of catfishes of the genus "Arius" (Siluriformes) ; *Curr. Sci.* 48 (22) 1008
- Ginsburg I 1938 Arithmetical definition of species, subspecies, and race concept with a proposal for a modified nomenclature ; *Zoologica* 23 253-286
- Dice L R and Leraas H J 1936 A graphic method for comparing several sets of measurements, *Contribs. Lab. Vertebr. Genetics, Univ. Mich. Ann. Arbor.* 3 3
- Hubbs C L and Perlmutter A 1942 Biometric comparison of several samples with particular reference to racial investigations ; *Amer. Nat.* 76 582-592
- Hubbs C 1952 A contribution to the classification of the blennoid fishes of the family Clinidae with a partial revision of the eastern pacific forms ; *Stanford ichthyol. Bull.* 4 (2) 41-165
- Jayaram K C and Dhanze J R 1978a Siluroid fishes of India, Burma and Ceylon. 21. A note on the systematic position of *Tachysurus serratus* (Day) (Ariidae) ; *Bull. zool. Surv. India* 1 (2) 203-205
- Jayaram K C and Dhanze J R 1978b Siluroid fishes of India, Burma and Ceylon 22. A preliminary review of the genera of the family Ariidae (Pisces : Siluroidea) ; *Matsya* 4 42-51.

- Jayaram K C and Dhanze J R 1981 Siluroid fishes of India, Burma and Ceylon. 23. The specific status of *Tachysurus malabariacus* (Day) (Ariidae); *Bull. zool. Surv. India* 4(1) 121-123
- Misra K S 1976 *The fauna of India and the adjacent countries*, Pisces, 3 xxi + 349 pp, 2nd ed.
- Munro I S R 1955 *The marine and Freshwater fishes of Ceylon*, Canberra, xvi + 349 pp.
- Pearl R 1930 *Introduction to Medical Biometry and Statistics*, Philadelphia and London
- Pillay T V R 1951 A morphometric and biometric study of the systematics of certain allied species of the genus *Barbus* Cuv. and Val.; *Proc. natn. Inst. Sci. India* 17 (5) 331-348
- Smith H M 1945 The freshwater fishes of Siam or Thailand; *Bull. U.S. Natn. Mus.*, Washington (188), xi + 622 pp.
- Smith J L B 1962 Fish from the cape described by Liethenetein 1833; *S. Afr. J. Sci.* 58 39-40
- Simpson G G and Roe A 1939 *Quantitative zoology*, New York
- Simpson G G, Roe A and Lewontin R C 1960 *Quantitative zoology* New York, Rev. ed.
- Snedecor G W 1956 *Statistical methods applied to experiments in agriculture and biology*, 5th Ed. Anus Iowa, Iowa State Collge Press
- Taylor W R 1978 *FAO species identification sheets for fishery purposes Western Central Atlantic* (Fishing Area 31) Rome 1
- Weber M and de Beaufort L F 1913 *The fishes of the Indo-Australian Archipelago* Leiden 2, xx + 404 pp.
- Winterbottom R 1980 Systematics, osteology and phylogenetic relationships of fishes of the osteriophysan subfamily Anostominae (Characoidei, Anostomidae); *Life Sciences Contribution Royal Ontario Museum* 123 Canada
- Wongratana T and Bathia U 1974 *FAO species identification sheets for fishery purposes Eastern Indian Ocean* (Fishing area 71) Rome 1

Graphs I-XX. Dice diagram showing the intergradation and divergence in respect of 20 characters in the four species of the genus *Arius*. In each diagram, the horizontal base line indicates the extreme range; the vertical line in the middle represents the arithmetic mean; the solid area on either side of the mean is the extent of one standard error; the hollow area delimits one standard deviation on either side of the mean; the hatching lines represent the extent of standard deviation beyond the extreme range.

Electron microscopic study of the spermatheca of *Gesonula punctifrons* (Acrididae: Orthoptera)

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Abstract. The present transmission electron microscopic study of the spermatheca of a common Indian grasshopper, *Gesonula punctifrons*, has highlighted the presence of the glandular secretory cells (SGC) and ductule cell (DC) in the spermathecal epithelium and additionally the occurrence of muscle cells, tracheoles and haemocytes. Both the former cell types are secretory in nature and probably their discharges in the lumen of the cuticle-lined spermathecal duct or ductule vary in their chemical nature. The ultrastructural evidence gives ample support to a concept of a lysosomal control of the secretory materials prior to their liberation in the lumen. The characteristic features of the plasma membranes of the secretory cells clearly suggest their involvement in the transepithelial transport of ions and smaller molecules across the basement membrane. A neuronal supply to the spermathecal wall is yet to be demonstrated to explain the filling in and out of the male gametes by this organ.

Keywords. Transmission electron microscope; spermathecal gland cell; ductule cell; rough endoplasmic reticula; plasma membrane; secretory granule; microvilli; nucleus; euchromatin; heterochromatin; male gamete; muscle cell; tracheole; haemocyte.

1. Introduction

Pal and Ghosh (1981) have described earlier the cytological, histological and histochemical features of the spermatheca of *Gesonula punctifrons*. The presence of glandular cells in the spermatheca of several insects has been documented (Imms 1957; Wigglesworth 1965; Adiyodi and Adiyodi 1975). According to Adiyodi and Adiyodi (1975), 'spermathecal cells have the capacity to resorb excess germ cells'. In recent years, conventional transmission electron microscopy (TEM) and scanning electron microscopy (SEM) have been employed to study the spermathecae of insects like, *Aedes aegypti* (Clements and Potter 1967; Jones and Fischman 1970), *Sitophilus granarius* (Tombes and Roppel 1971), *Dytiscus marginalis* (Conti et al 1972), *Apis mellifera* (Dallai 1975), *Drosophila melanogaster* (Filosi and Perotti 1975), *Tenebrio molitor* (Happ and Happ 1975), etc. These studies have raised issues with regard to the homology and analogy of the spermathecae in different insects (Huebner 1980), the nature of secretion (Copland and King

1972; Filosi and Perotti 1975), the possible role in oogenesis (Dumser 1969; Boulétreau-Merlé 1977), etc. The present paper would provide a comprehensive description of the spermathecal epithelium of *G. punctifrons* as seen under the TEM.

A detailed account on the fine structural morphology of the spermathecal epithelium, musculature, tracheoles and innervation is necessary before forwarding an explanation for the mechanism(s) of controlled movement of spermatozoa into and out of the spermathecae. The mode or style of functioning of the spermathecae either in the yellow fever mosquito, or in the Chalcids or in cockroaches is not free from confusion (Jones and Wheeler 1965a, b; Dent 1970; Jones and Fischman 1970; Copland and King 1972).

According to Pal and Ghosh (1981) the spermathecal epithelial cells of *G. punctifrons* are highly active and secrete copious amount of mucoprotein. Usually osmophilic and PAS-positive materials appear juxta-nuclearly and these coalesce apically to be subsequently transported to the lumen of the spermatheca by means of cuticle lined ductules (Clements and Potter 1967; De Camargo and Mello 1970; Poole 1970). It has been claimed by Lensky and Allmot (1969) that some fractions of the haemolymph proteins migrate to the spermathecal fluid in honey-bee. An alternative route of release of some unknown spermathecal contents or factors responsible for the growth of the female gonad in insects has been suggested by Dumser (1969) and Boggs and Gilbert (1979).

According to Huebner (1980) the ultrastructural features of the spermathecae of *Rhodnius prolixus* differ remarkably from those of other insects studied so far. The characteristic presence of apically situated secretion-loaded tubular invagination or invagination in the glandular cells is a novelty and is not represented widely. Furthermore, this apical cone lacks a cuticular lining. Therefore, it is suggested that a systematic inventory on the similarities and dissimilarities of the spermathecal epithelium in insects may indicate its true nature in reproduction, its relation to other ectodermal invaginations/glands etc. Gupta and Smith (1969) showed in *Periplaneta americana* the presence of nerves and myoneural junctions in the striated muscles of spermathecae which could be analogous to the myoepithelium of newt spermathecae (Dent 1970). A future publication would include the mapping of the fine structural details of the muscle cells, haemocytes and the nerve supply (?) of the spermathecae of *Gesonula punctifrons*.

2. Materials and methods

Spermathecae from the adult female grasshoppers were dissected in insect Ringer solution and small pieces were fixed in 1% ice-cold glutaraldehyde in 0.1 M phosphate buffer (pH 7.2-7.4) for one hour (Sabatini *et al* 1962) at 4°C. Subsequently these were washed twice in the buffer and post-fixed in 1% OsO₄ in distilled water at room temperature for two hours. After double fixation, tissue pieces were dehydrated in ethanol with or without uranyl acetate and embedded in plastic capsules with araldite mixture (Luft 1961). Ultrathin sections were cut on a LKB-ultratome with glass-knives and stained with uranyl acetate and lead citrate (Reynolds 1963) and viewed under a transmission electron microscope (Siemens Elmiskop I) with naked copper grids.

3. Observations

Figure 1 gives the essential features of the spermathecal glandular cells (SGC) and the peri-luminal ductule cells (DC). The glandular epithelial cells (SGC) of the spermatheca of *Gesonula punctifrons* rest on a thick basement membrane (BM) which is supported by an underlying layer of deep striated muscles (MC) and supplied by fine branches of the tracheoles (T) (figure 2). The tracheoles have a diameter 0.3μ . The basal plasma membranes of these cells make extensive and characteristic infoldings, while the lateral plasma membranes run slightly unevenly leaving a minimum of intercellular space. The entire thickness of two lateral plasma membranes and the space between them is 340 \AA . Apically the cell membrane forms numerous brush-border like processes around the cuticle-lined lumen of the spermathecae. The nuclei (N) are large (10μ in diameter), covered by a double-layered nuclear envelope (NE). The outer leaflet of the nuclear envelope is studded with ribonucleo-protein particles. Frequently, a medium-sized nucleolus (NCL) is observed within the nucleus. The entire nucleoplasm shows uniform but moderate electron density. However, small but regularly-sized dense-particles are observed at the boundary between the nucleolus and the

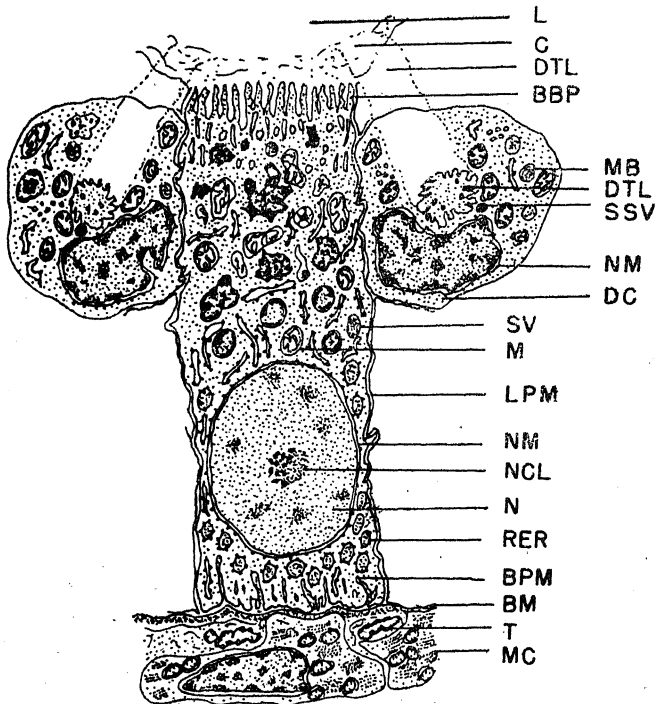


Figure 1. Semi-diagrammatic representation of the ultrastructural features associated with the spermathecal glandular cell (SGC) and the ductule cell (DC) of *Gesonula punctifrons*. Both muscle cells (MC) and tracheoles (T) surround the spermathecal epithelium.

nucleoplasm. These dense intranucleolar RNP (?) particles measure about 300 Å in diameter (figure 3).

The cytoplasm is full of rough surfaced endoplasmic reticula (RER), several of which appear in the form of ovoid or spherical bodies containing electron dense materials. These measure about $0.3\ \mu$ in diameter. Associated with these small bodies there occur many medium-sized membrane-bound dense bodies with slightly granular peripheral zones. These measure about $0.9\ \mu$ in diameter. The rest of the contents of these bodies has similar electron opacity as those of the RER vesicles. Supranuclearly the cytoplasm contains many membrane-delimited pleomorphic entities (figure 2). These are filled with a granular matrix, corpuscular dense microstructures, membranous profiles and microvesicles. Some of these bodies also occur in the apical cytoplasm of the glandular cells of the spermatheca. There are several round or ovoid mitochondria (M) containing relatively fewer cristae.

Topographically the ductule cells (DC) appear in groups surrounding the lumen (L) of the spermatheca (figures 1 and 4). Further, the ductule cells (DC) are mononucleated cells which are characterized by the dense nuclei with irregular outlines. Both densely-staining heterochromatin and lightly staining euchromatin are present within the nuclei of these cells. The nuclear envelope has a thickness of 300 Å. Nucleolus may be present or absent. Occasionally pores on the nuclear envelope are observed. The cytoplasm of these cells is characterized by the presence of numerous large rounded mitochondria and myelin bodies (MB) having a diameter $0.5\ \mu$. Myelin bodies consist of whorls of fine membranes. Near the lumen the cell apices are thrown into numerous microvilli which surround the ductule. A single microvillious process has a diameter of $0.1\ \mu$. Laterally the plasma membranes show extensive interdigitations and folds to increase the surface area, though the intercellular space is exceedingly delimited. The total width of two lateral plasma membranes and the intercellular space varies from 300 to 350 Å (figure 4). Besides, the cytoplasm has fewer ribosomes, RER and SER. A few vesicles with varying contents are usually observed in these cells.

The paragonadal haemocoel is filled with numerous haemocytes, muscle cells and the tracheoles. The haemocytes possess prominent rounded nuclei with numerous cytoplasmic granules (figure 5). There is only a particular haemocyte which is very common around the spermatheca of *Gesonula punctifrons*. These are rounded or rarely irregularly outlined cells occurring between the muscle cells and the tracheoles reaching the spermathecal wall. Infrequently binucleate haemocytes are observed (figure 6). Usually the nucleus is $7\ \mu$ in diameter. Both euchromatin and heterochromatin are distinguished in these nuclei. The cytoplasm is populated by three different types of granules: (a) small, dense bodies, measuring approximately 500 Å in diameter, (b) intermediate type of dense granules measuring about $1.2\ \mu$ in diameter and (c) a larger variety of $2.5\ \mu$ in diameter. These three classes of intracytoplasmic granules are uniformly distributed within the haemocyte. The larger type of granules have characteristic electron-lucent zone of separation between their membranes and the moderately granular contents. The rest of the cytoplasm is occupied by mitochondria and rough-surfaced endoplasmic reticula. A clear-cut Golgi apparatus has not been seen in these preparations.

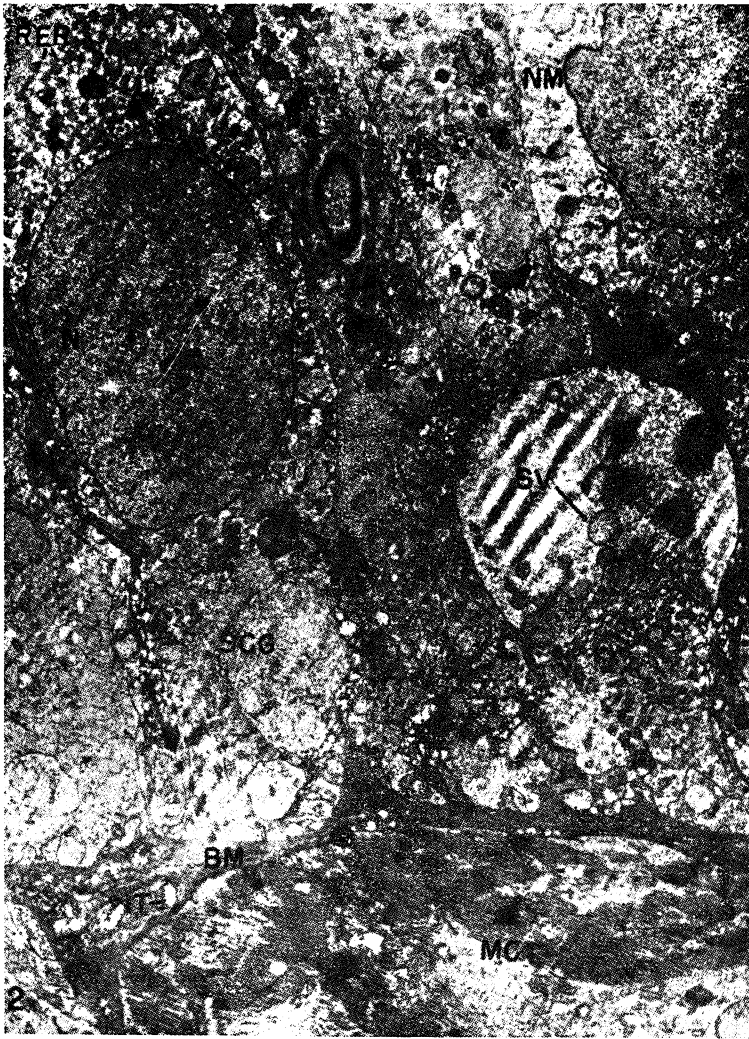


Figure 2. A low magnified electron microphotograph of the spermathecal glandular cells (SGC) detailing the different cellular components. Presence of numerous RER vesicles and a large nucleus is a constant feature. $\times 6,500$.

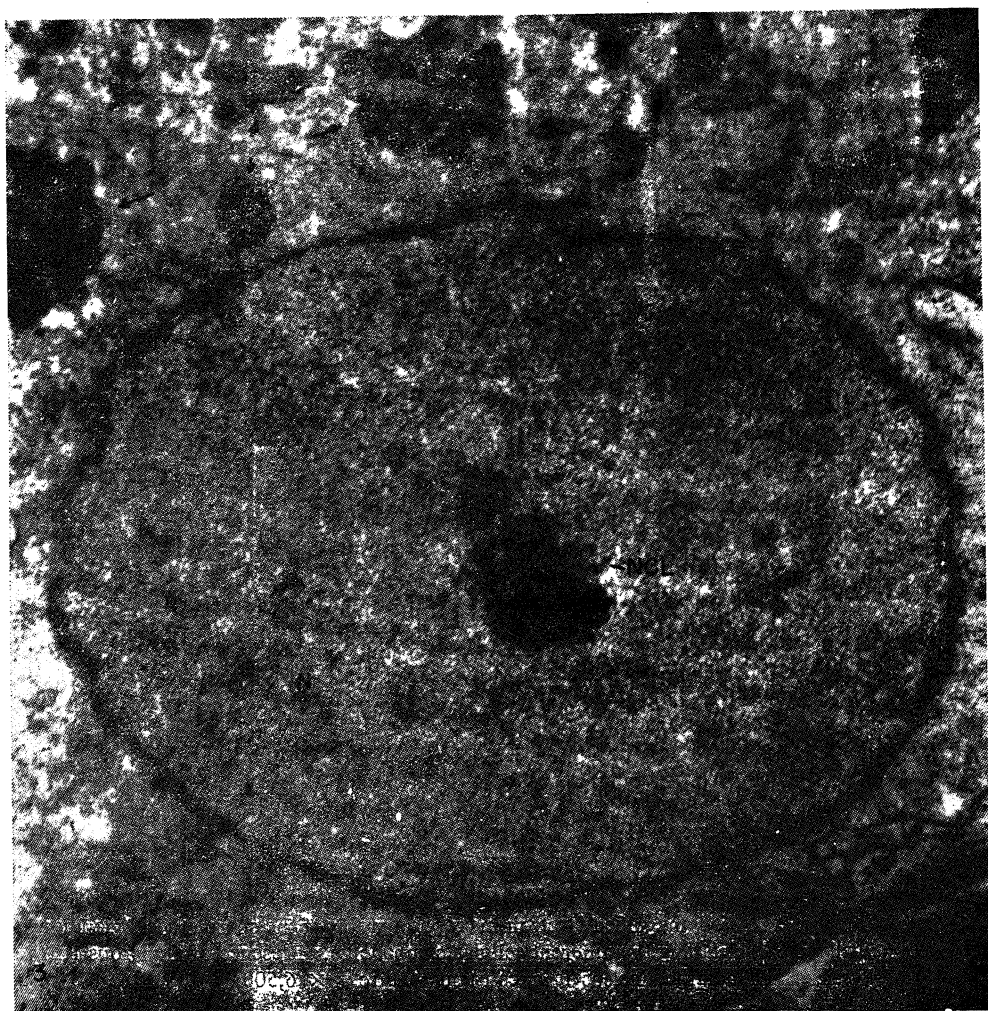


Figure 3. A large electron micrograph of the nucleus (N) and the perinuclear cytoplasm of the SGC showing nucleolar peculiarities and the cytoplasmic large dense pleomorphic bodies (arrows). $\times 16,000$.

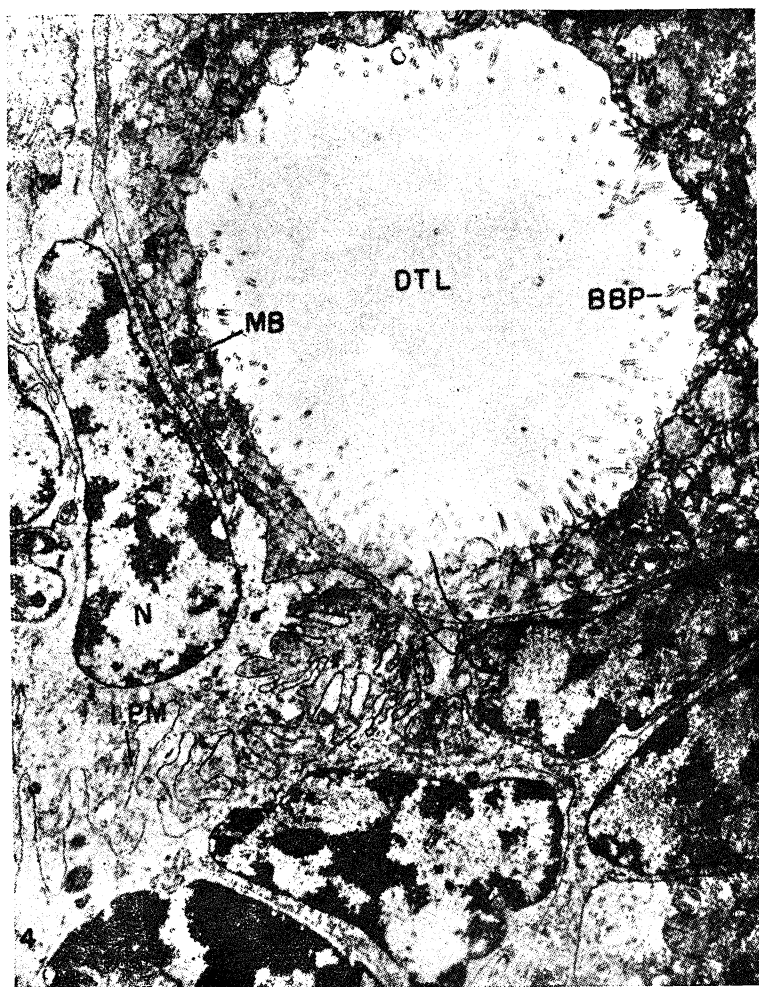


Figure 4. Electron micrograph detailing the nucleated ductule cells surrounding the lumen (DTL), the interdigitating lateral plasma membranes (LPM) and myelin bodies (MB). $\times 6,000$.



Figure 5. Haemocytes (H) and muscle cells (MC) populate the para-spermathecal coelome of grasshoppers. Fine structures of haemocytes and in particular the cytoplasmic granules (CG) are apparent in this micrograph. $\times 3,200$.



Figure 6. Electron micrograph detailing the ultrastructural features of a binucleate haemocyte (H). $\times 3,200$.

4. Discussion

According to Adiyodi and Adiyodi (1975) and Huebner (1980) the glandular cells of the spermatheca are characterized by lateral interdigitations between the adjacent cells and the basally situated large ovoid nuclei. Besides, ribosome rich cytoplasm in *Aedes aegypti* (Clements and Potter 1967) or basophilic cytoplasm in *Apis mellifica* (De Camargo and Mello 1970 ; Poole 1970) is an essential feature for the protein synthesizing glandular cell in the spermatheca of insects. The importance of a large nucleus with prominent nucleolus, numerous rough endoplasmic reticula, Golgi apparatus, etc. are well-known for cellular protein synthesis (Palade *et al* 1962). We have not observed a Golgi apparatus in the spermathecal gland cells of *G. punctifrons* but this does not mean that it is definitely absent. Huebner (1980) has described the presence of Golgi apparatus in the spermathecal epithelium of *Rhodnius prolixus*.

This paper records the occurrence of two distinct cell types in the epithelium of the spermatheca of an Indian grasshopper. Moreover, the fine structural differences between these cells and their topographic distributions clearly suggest their long morphogenetic separation and functional specialization. Both these cells are partly or entirely secretory and glandular but they sharply differ in several features and probably also in their secretory products. The mechanism of release or drainage of the secretory products by these cell types presumably differ due to the presence of a cuticle-free ductule in one case and the presence of extensive apical plasmalemmal infoldings in the large glandular cell. Apparently there is no comparable organelle as that of the apical invagination as described by Huebner (1980) for *Rhodnius prolixus*. However, it is not clear whether the intracellular canaliculi of *Apis mellifica* ideally correspond with the cuticle-lined ductule observed by several workers (Copland and King 1972). The ductule cells of the spermatheca of *G. punctifrons* are somewhat low with prominent lobate nuclei containing dense chromatin masses. There are swarms of mitochondria around the microvilli of these cells. The lumen is cuticle-free. The cytoplasm possesses several small-sized, membrane-bound dense microstructures containing secretory materials. Apart from these, there also occur a few myelin bodies, cells release their products through the smooth-surfaced microvesicles to the ductule, the lumen of which demonstrate the presence of moderately electron dense granular substances. It is likely that these cells liberate chemically different substances from the true spermathecal glandular cells (SGC). There are, however, claims that spermathecae in different insects liberate dissimilar chemical moieties to their lumen (Clements and Potter 1967 ; Bhatnagar and Musgrave 1971 ; Filosi and Perotti 1975). The secretory products may be mucoprotein or lipoprotein. It is still unsettled whether a similar situation exists in a species of insect. The discharge of a sperm-activating factor from the spermatheca of Eurytomidae has been reported by Copland and King (1972).

On the contrary, the spermathecal glandular cells of grasshoppers are tall epithelial cells with narrow width and a large ovoid basal nucleus. The basal plasma membrane of these cells show deep and elaborate infolding. These may be involved in active transport of ions and probable absorption of some protein fractions of the haemolymph. Similar claims have been separately reported in *Periplaneta americana* (Gupta and Smith 1969) and in *Apis mellifera* (Lensky and

Allmot 1969). A minimum of intercellular space over the lateral plasma membranes is suggestive of a low diffusion of ions and small molecules. However, this cell gives an unmistakable evidence for the synthesis of proteins, likely to be transported to the lumen of the spermatheca. Numerous RER vesicles gradually enlarge and store granular materials. These slowly loose the surface ribosomes from their membranes and attain peripheral condensations as they enlarge to form medium and large sized secretory spheres or droplets. Supranuclearly the cytoplasm shows the presence of a different class of pleomorphic organelles. It remains to be resolved in future whether these entities are the later developmental stages of the secretory spheres or an entirely new class of sub-cellular bodies or vesicular organelles as described by Copland and King (1972). However, both these classes of the secretory bodies move apically and ultimately release their contents by means of exocytosis (?) near the apical foldings to the spermathecal lumen. It is hardly known how the intracellular transport and the direction of the secretory products, etc., are regulated by means of the microtubules as reported by Huebner (1980) or by the intervention of a lysosome system to control the overproduction of the secretory materials (Smith and Farquhar 1966).

Jones and Fischman (1970) have given the ultrastructural details of the plasmotocytes occurring in the vicinity of the spermathecal complex of *Aedes aegypti*. Our description of the haemocytes from the para-spermathecal haemocoel of the grasshoppers is suggestive of their granular nature; and additionally the presence of a large ovoid nucleus and the absence of the pseudopodial extensions justify that these belong not the plasmotocytic cell type. Again the spherule cells and the cytotocytes both possess granules which enclose several microtubular profiles (Ratcliffe and Price 1974). These workers have further suggested that it is difficult to clearly identify the various haemocytes of the insects under both the light and the electron microscope. However, it is extremely premature at this stage to indicate the active participation of these haemocytes in the adult reproductive structures of insects.

From the data presented here and also from those reported earlier (Jones and Fischman 1970 ; Huebner 1980 ; Pal and Ghosh 1981) it is abundantly clear that the spermathecal epithelium varies quite strikingly from the distal portion to the duct. But in *Gesomula punctifrons* the spermathecal gland cells (SGC) have unique distribution both in the proximal and distal regions as well as in the duct zone. An extensive examination of the sectioned materials both with light and transmission electron microscope (TEM) may provide information on the histological and subcellular transitions in the spermatheca of grasshoppers. This could be extended to include the cuticular intima, muscles, haemocytes, tracheoles and nerves so that a comprehensive account on the insect spermatheca may be established.

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References

- Adiyodi K G and Adiyodi R G 1975 Morphology and cytology of the accessory sex glands of invertebrates; *Int. Rev. Cytol.* **43** 353-398
- Bhatnagar R D S and Musgrave A J 1971 Aspects of histophysiology of the spermathecal gland of *Sitophilus granarius* (L.) (Coleoptera); *Can. J. Zool.* **49** 275-277
- Boggs C and Gilbert L 1979 Male contribution to egg production in butterflies: Evidence for transfer of nutrients of mating; *Science* **206** 83-84
- Boulétreau-Merlé J 1977 Role des spermatheques dans l'utilisation du sperme et la stimulation de l'ovogenese Chez *Drosophila melanogaster*; *J. Insect Physiol.* **23** 1099-1104
- Clements A N and Potter S A 1967 The fine structure of the spermathecae and their ducts in the mosquito *Aedes aegypti*; *J. Insect Physiol.* **13** 1825-1836
- Conti L, Ciofi-Luzzatto A and Autori F 1972 Ultrastructural and histochemical observations on the spermathecal gland of *Dytiscus marginalis* L. (Coleoptera); *Z. Zellforsch.* **134** 85-96
- Copland M J W and King P E 1972 The structure of the female reproductive system in the Eurytomidae (Chalcidoidea: Hymenoptera); *J. Zool.* **166** 185-212
- Dallai R 1975 Fine structure of the spermatheca of *Apis mellifera*; *J. Insect Physiol.* **21** 89-109
- De Camargo J M F and Mello M L S 1970 Anatomy and histology of the genital tract, spermatheca, spermathecal duct and glands of *Apis mellifica* queens (Hymenoptera: Apidae); *Apidologie* **1** 351-373
- Dent J N 1970 The ultrastructure of the spermatheca in the red spotted Newt.; *J. Morphol.* **132** 397-424
- Dumser J B 1969 Evidence for a spermathecal hormone in *Rhodnius prolixus* (Stal); M.Sc. Thesis, Biology Department, McGill University, Montreal, Quebec, Canada
- Filosi M and Perotti M E 1975 Fine structure of the spermathecae of *Drosophila melanogaster* Meig; *J. Submicrosc. Cytol.* **7** 259-270
- Gupta B L and Smith D S 1969 Fine structural organization of the spermatheca in the cockroach *Periplaneta americana*; *Tissue Cell* **1** 295-324
- Happ G M and Happ C M 1975 Fine structure of the spermatheca of the milkworm beetle (*Tenebrio molitor* L.); *Cell Tissue Res.* **162** 253-269
- Huebner E 1980 Spermathecal ultrastructure of the insect *Rhodnius prolixus*; *J. Morphol.* **166** 1-25
- Inms A D 1957 *A general text-book of entomology* (London: Methuen) p. 325
- Jones J C and Fischman D A 1970 An electron microscopic study of the spermathecal complex of virgin *Aedes aegypti* mosquitoes; *J. Morphol.* **132** 293-312
- Jones J C and Wheeler R E 1965a Studies on the spermathecal filling in *Aedes aegypti* (Linnaeus). I. Description; *Biol. Bull.* **129** 134-150
- Jones J C and Wheeler R E 1965b Studies on the spermathecal filling in *Aedes aegypti* (Linnaeus). II. Experimental; *Biol. Bull.* **129** 532-545
- Lensky Y and Alumot E 1969 Proteins in the spermathecae and haemolymph of the queen bee (*Apis mellifica* L. var. *lingustica* Spin); *Comp. Biochem. Physiol.* **30** 569-575
- Luft J H 1961 Improvements in epoxy resin embedding methods; *J. Biophys. Biochem. Cytol.* **2** 409-414
- Palade G E, Siekevitz P and Caro L G 1962 *Structure, chemistry and function of the pancreatic exocrine cell*; Ciba Foundation Symposium on the Exocrine Pancreas (London: J and A Churchill Ltd.) pp. 23-49
- Pal S G and Ghosh D 1981 Functional histomorphology of the spermatheca of an Indian grasshopper, *Gesonula punctifrons* (Acrididae: Orthoptera); *Proc. Indian Acad. Sci. (Anim. Sci.)* **90** 161-171

- Poole H K 1970 The wall structure of the Honey Bee Spermatheca with comments about its function ; *Ann. Ent. Soc. Am.* 63 1625-1628
- Ratcliffe N A and Price C D 1974 Correlation of light and electron microscopic hemocyte structure in the Diptera ; *J. Morph.* 144 484-498
- Reynolds E S 1963 The use of lead citrate at high pH as an electron opaque stain in electron microscopy ; *J. Cell Biol.* 17 208-212
- Sabatini D D, Bensch K G and Barnett R J 1962 New Fixatives for Cytological and Cytochemical Studies. Proc. 5th Intern. EM. Cong. Vol. 2, L-3, Philadelphia
- Smith R E and Farquhar M G 1966 Lysosome function in the regulation of the secretory process in cells of the anterior pituitary gland ; *J. Cell Biol.* 31 319-347
- Tombs A S and Roppel R M 1971 Scanning electron microscopy of the spermatheca in *Sitophilus granarius* (L.) ; *Tissue Cell.* 3 551-556
- Wigglesworth V B 1965 *The principles of insect physiology* (London : Methuen)

Abbreviations

BBP	..	Brush border processes
BM	..	Basement membrane
BPM	..	Basal plasma membrane
C	..	Cuticle
CG	..	Cytoplasmic granule
DC	..	Ductule cell
DTL	..	Ductule lumen
H	..	Haemocyte
L	..	Lumen of the spermatheca
LPM	..	Lateral plasma membrane
M	..	Mitochondrion
MB	..	Myelin body
MC	..	Muscle cell
N	..	Nucleus
NCL	..	Nucleolus
NM	..	Nuclear membrane
RER	..	Rough endoplasmic reticulum
SGC	..	Spermathecal glandular cell
SV	..	Secretory vesicle
SSV	..	Small smooth surfaced microvesicle
T	..	Fine tracheole.

Histology and histochemistry of adrenal glands of Indian mongoose *Herpestes edwardsii edwardsii* (Geoffroy)*

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Abstract. The histology of the adrenals of the mongoose *Herpestes edwardsii edwardsii* has been studied. Three layers in the cortex, namely zona glomerulosa, zona fasciculata and zona reticularis and central medulla surrounded by the cortex have been observed.

Employing histochemical techniques it was revealed that the cortex is rich in glycoproteins, lipids and protein bound Amino groups. It has moderate amounts of proteins containing sulphhydryl and disulphide radicals and tyrosine. Tryptophan has not been detected.

Negligible amounts of mucopolysaccharides were detected in the medulla. Aspects dealing with the occurrence of carbohydrates, proteins and lipids in various regions of the cortex are discussed.

Keywords. Histology ; histochemistry ; adrenal glands ; *Herpestes edwardsii edwardsii*.

1. Introduction

Studies on the smaller terrestrial mammals have been generally confined to rodents. Carnivores seem to have been neglected probably due to the difficulties encountered in their collection and rearing. Histology of adrenal glands have been studied by Meyers and Charipper (1956), Pauly (1957), Holmes (1961), Houser *et al* (1962) and McKeever and Tomich (1963). Hunt and Hunt (1959) studied the glycogen content in the adrenal glands of rats at different ages and a detailed account of glycogen in adrenals was furnished by Girod (1960). Sinha and Ghosh (1961) gave information on the adrenal cortical cytochemistry in the pigeon. Prasad and Yadav (1974) made observations on the histological and histochemical details of the adrenal glands of the Indian buffalo. Recently Carole *et al* (1979) studied the histological details of adrenals in newborn alpaca. Our knowledge of the adrenal glands of carnivorous wild mammals is meagre. In this paper an attempt

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has been made to bring out histological and histochemical aspects of the adrenal glands of the Indian mongoose *Herpestes edwardsii edwardsii*.

2. Materials and methods

Mongoosees were obtained from villages nearby Visakhapatnam town and were acclimatized to laboratory conditions. Adrenal glands were removed from the animal and were fixed in Zenker or Bouin's or Susa or formol-calcium. After routine procedures of dehydration and embedding, 5 to 7 μ thick sections were cut. Heidenhain's Azan, Mallory's triple stains were employed to study the histological details. The histological and histochemical techniques were adopted from Gomori (1952), Lillie (1954), Carleton and Drury (1957), McManus and Mowry (1960), Gurr (1962), Barka and Anderson (1963), Humason (1965), Pearse (1968), Culling (1974) and Bancroft (1975).

3. Observations

Anatomically the adrenals of mongoose appear quite regular in shape. The left gland is relatively long and flattened whereas the right one is thick with latero-ventral angular borders. Both left and right adrenals lie closely pressed to the dorsal body wall anterior to the kidneys. The caudate lobe of the liver envelopes the right gland whereas the left one is free and is lightly pressed by the pancreas and stomach.

Two regions could be distinguished in the adrenals the outer cortex and central medulla. The gland is ensheathed by thin fibrous capsule. The cortex has 3 layers, the outer zona glomerulosa, middle zona fasciculata and an inner zona reticularis.

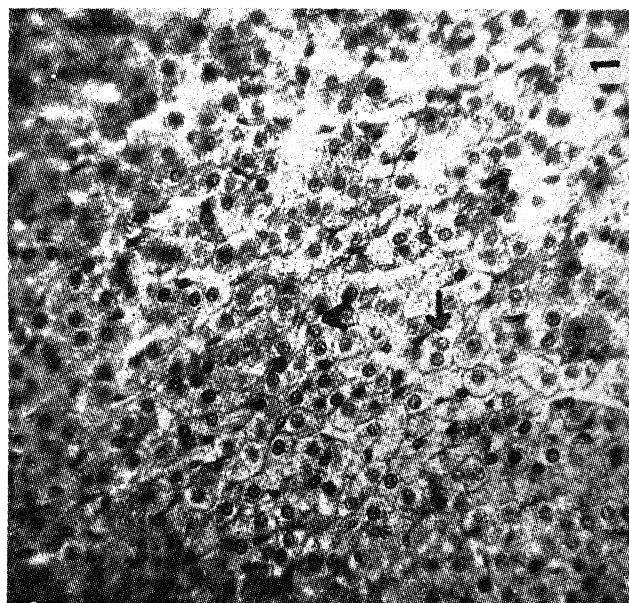
The capsule is formed by a combination of collagenous, elastic and reticular fibres. Smooth muscle fibres are also associated with the connective tissue.

The zona glomerulosa has a cellular structure and is delineated from the capsule on the outer side and the zona fasciculata from the inner side. The cytoplasm is basophilic in nature. In this zone the cells are more columnar and arranged in vertical single rows. The cells with a single nucleus which have usually one nucleolus each but some with double nucleoli could be seen occasionally.

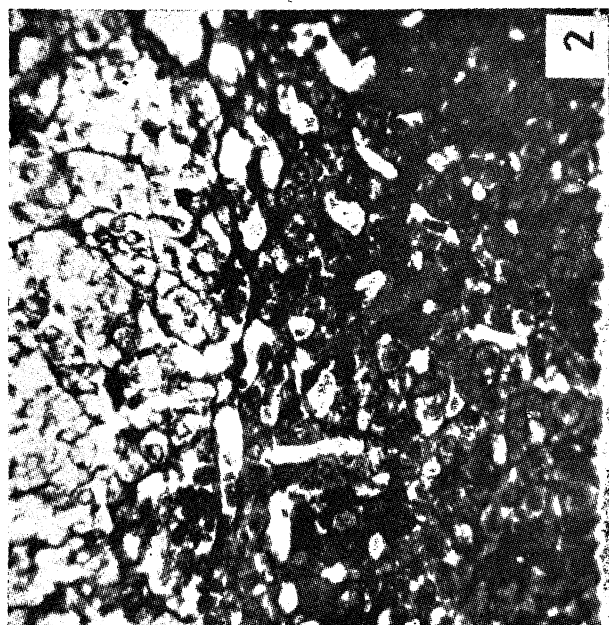
The zona fasciculata is the major portion of the cortex with cuboidal cells and some columnar cells. The cells are polygonal in shape and arranged in radiating columns. The cytoplasm is homogeneous and the nucleus is spherical and centrally situated. The size of the nuclei increases progressively towards the medullary part. The cells and their nuclei are larger than those of zona glomerulosa. The cells usually display a single nucleus with a nucleolus, but double nucleated cells also occur (figure 1).

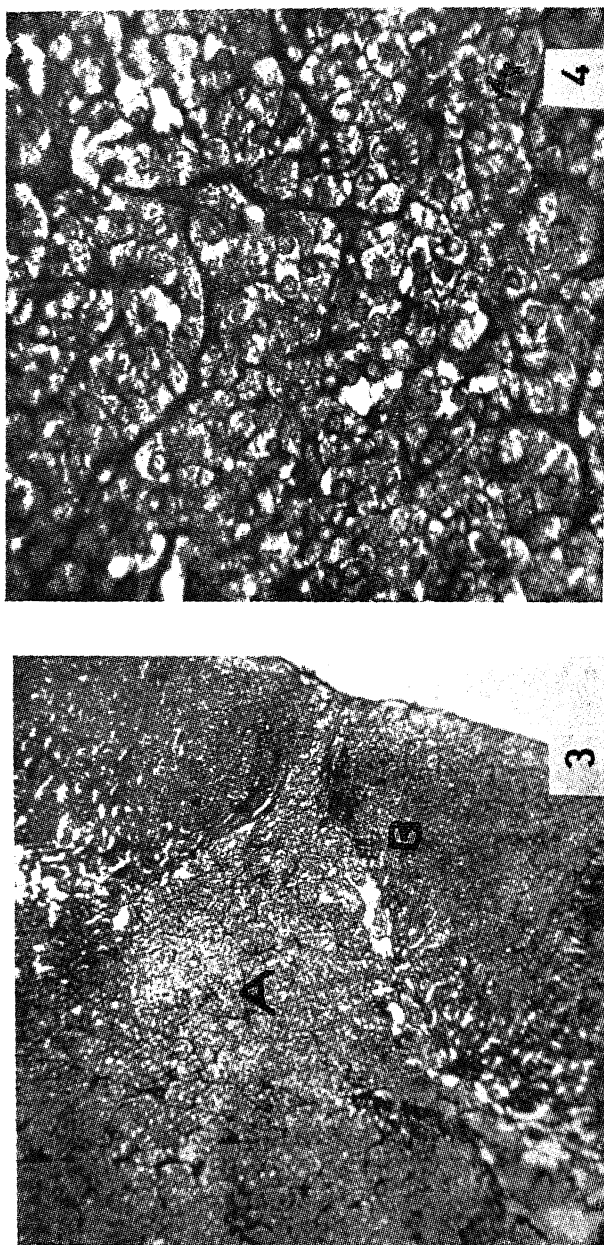
The histological details of zona reticularis are in agreement with those described for other mammals. This region is interspersed with sinusoids of various sizes giving the appearance of a broken network.

To make a clear-cut demarcation between the zona fasciculata and zona reticularis is rather difficult (figure 2). Zona reticularis is well developed in adults than in young ones and a distinct demarcation between the cortex and medulla is notice-



Figures 1-2. 1. Zona fasciculata at a higher magnification showing c.II with two nuclei (arrow) and polygonal cell (A) (5×40). 2. Portion of adrenal gland showing zona fasciculata and zona reticularis (5×40).





Figures 3-4. 3. Medulla protruding into zona fasciculata, A—medulla; B—zona fasciculata (5×40). 4. Medulla (5×40).

lable in adult animals. There is a mixing up of medullary cells with reticularis cells, but the extension of these cells is limited to zona fasciculata only (figure 3).

The medullary cells are arranged in irregular rows and the cells are smaller than those of cortical cells. The cells are arranged in irregular groups of 2 to 10 and mainly surrounded by thick strands of interlocking connective tissues and are lightly stained with histological stains when compared to cortex (figure 4).

4. Histochemical observations

The medulla in general gives a moderate reaction with bromophenol blue and Millon's reactions but tryptophan and arginine are absent as evidenced by negative response to *p*-dimethylaminobenzaldehyde nitrite and Sakaguchi reactions respectively (table 1). Medulla is moderately positive to ninhydrin/Schiff and chloramine-T/Schiff when compared to other protein reactions such as KMnO_4/AB , ferric ferricyanide indicating that large amounts of protein bound amino groups rather than disulphides and sulphhydryls, are present. The medulla is positive to lipids (Sudan black B) and phospholipids (copper phthalocyanin). With Congo red it stains moderately indicating the presence of glycoproteins.

Table 1. Histochemical reactions of the adrenal glands.

Test applied	Medulla	Zona glomerulosa	Zona fasciculata	Zona reticularis	Capsule
PAS	+	++	++	++	+++
PAS/Acetylation	+	++	++	++	+++
PAS/Deacetylation	+	++	++	++	+++
PAS/Methylation	+	+	+	++	++
Schiff's alone	+	+	+	+	+
Alcian blue-1 pH	±	±	±	±	±
Alician blue-2.5 pH	+	+	+	+	+
Congo red	++	+	++	+	+++
Bromophenol blue	++	++	++	++	++
BPB/Vanslykes	-	-	-	-	-
Millon's reaction	++	++	++	++	+++
DMAB/Nitrite	-	-	-	-	-
Sakaguchi	-	-	-	-	-
$\text{KMnO}_4/\text{alcian blue}$	+	++	+	++	++
Ninhydrin-Schiff	++	++	+++	++	+++
Chloramine-T/Schiff	++	++	+++	++	+++
Ferric ferricyanide	+	++	++	++	++
Sudan black B	++	+++	++	++	+++
Copper phthalocyanin	++	++	+++	++	+++

+++ = Strongly positive ; ++ = Moderately positive ; + = Faintly positive ;
- = Negative.

Zona glomerulosa is moderately positive to all protein tests showing their presence in small quantities. Protein bound amino groups and basic proteins like tyrosine are present in little amounts, but tryptophan and arginine are absent. This is the lipid rich part of the cortex. Its intense staining with Congo red indicates that it is rich in glycoproteins. But negligible amounts of Mucopolysaccharides are noticed. The zona fasciculata is rich in protein bound amino groups but tyrosine is present only in moderate amounts. This part of the cortex like other parts is devoid of tryptophan and arginine. Moderate amounts of sulphhydryls and disulphides have been localised. Phospholipids are abundant in this area. No mucopolysaccharides have been detected, but glycoprotein is present in abundance.

The reticular zone is rich in tyrosine but is devoid of mucopolysaccharides or basic proteins containing arginine and tryptophan. The protein bound amino groups, sulphhydryls and disulphides are present in moderate amounts. As in the case of adrenals of other mammals, this region displays mild amounts of lipids.

5. Discussion

In the zona glomerulosa the presence of double nucleolated cells was also observed in the Indian buffalo by Prasad and Yadav (1974). In the ferret (Holmes 1961) and in the Indian buffalo (Prasad and Yadav 1974) it was observed that zona glomerulosa took lighter stain with histological stains than the cortical layers, an observation which is in agreement with the present findings. This may be due to glucocorticoids that are secreted by the zona glomerulosa which take lighter stain.

The presence of faintly stained cytoplasm in this zone also agrees with the condition reported by Meyers and Charipper (1956) for the golden hamster, by Hewer and Foster (1966) for man, Holmes (1961) for ferret and Houser *et al* (1962) for Panama monkeys.

McKeever and Tomich (1963) observed an arc of cells at the capsular end in *Herpestes auropunctatus* in mature females but this condition could not be seen in the present study. The zona fasciculata occupies the major portion of the cortex in mongoose as is the case in bulls (Cupps *et al* 1954; Das *et al* 1965) and in Indian buffalo (Prasad and Yadav 1974). This is attributed to the fact that this may be synthesizing and secretory zone for steroidal hormones. McKeever and Tomich (1963) reported that in *Herpestes auropunctatus* there is a clear demarcation between inner and outer fasciculata in sexually active female, which could not be corroborated in our study on *Herpestes edwardsii edwardsii*, as our observations were made on females in captivity. Estrogen secreting activity may augment the bulk and reactivity of zona fasciculata which actually forms a band of cells and could be considered the estrogen secreting zone. During the course of development this estrogen secreting zone may extend into zona glomerulosa side constituting distinct zona intermedia—a condition which occurs in Indian buffalo.

Progressive increase in size of the cells in deeper parts of zona fasciculata has been reported by Copenhaver (1964) in man and by Prasad and Yadav (1974) in Indian buffalo. The larger sizes of the nuclei and cells in zona fasciculata are in

agreement with the findings in bull and bullock (Cupps *et al* 1954; Sohal and Chaturvedi 1962). However, Hartman (1959) found that the cells of this zone were smaller than those of zona glomerulosa in the adrenal glands of the sloth.

The zona reticularis is comparatively better developed in adult animals than in young ones, thus agreeing with the observations of Prasad and Yadav (1974) in Indian buffalo. As far as mixing up of cells in the medulla is concerned Holmes (1968) also found this condition in *Macaca mulatta* but Prasad and Yadav (1974) state that medullary cells migrate up to the level of zona glomerulosa.

The medulla in general is rich in lipids obviously because of the principal secretions of medulla, adrenaline and noradrenaline. A positive ninhydrin/Schiff reaction is due to free amino group in adrenaline and noradrenaline. As a whole the cortex is strongly positive to lipid stains, since gluco and adrenocorticoids are lipids in nature. The cortex displays a comparatively more intense reaction for proteins. After deamination with van Slykes reagent cortex as well as medulla became negative to bromophenol blue and other basic protein tests.

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References

- Bancroft J D 1975 *Histochemical techniques* (London and Boston : Butterworths)
- Barka T and Anderson P J 1963 *Histochemistry theory, practical and bibliography*. Hoeber Medical Division (New York, Evanston and London : Harper and Row)
- Carleton H M and Drury R A B 1957 *Histochemical techniques for normal and pathological tissues and the identification of parasites* (London : Oxford University Press) pp. 1-343
- Carole A, Samuel J, Sumer and Nathanielz P W 1979 Histological observations of the adrenal glands of newborn alpacas (*Lama pacos*) ; *Comp. Biochem. Physiol.* A62 387-396
- Copenhaver W M 1964 *Bailey's text-book of histology*, 15th edn. (Baltimore : Williams and Wilkins) pp. 582-583
- Culling C F A 1974 *Hand-book of histopathological and histochemical techniques* (London and Boston : Butterworths)
- Cupps P T, Laben R C and Mead S W 1954 Histology of the pituitary, testis and adrenal in relation to reproduction in the bull ; *J. Dairy Sci.* 37 1074-1087
- Das L N, Mishra D B and B'swal G 1965 Comparative histological study of adrenal and thyroil glands of the bull and the bullock ; *Indian Vet. J.* 42 824-830
- Girod C 1960 Adrenal glycogen ; *C.R. Soc. Biol.* 154 2049-2051 (Vide *Excerpta Med.* 16 229 ; 1962)
- Gomori G 1952 *Microscopic histochemistry*, Chicago University Press
- Gurr H 1962 *Staining : Animal tissues, practical and theoretical* (London : Leonard Hill)
- Hartman F A 1959 Notes on the adrenal of the sloth ; *Anat. Rec.* 133 105-114
- Hewer E E and Foster C L 1966 *Hewer's text-book of histology for medical students* (London : William Heinmann Medical Books Ltd.) pp. 204-210
- Holmes R L 1961 The adrenal glands of the ferret, *Mustela putorius* ; *J. Anat.* 95 325-336
- Holmes R L 1968 The adrenal glands of *Macaca mulatta*, with special reference to the cortico-medullary zone ; *J. Anat.* 103 471-477

- Houser R G, Hartman F A, Knouff R A and McCoy F W 1962 Adrenals in some Panama monkeys ; *Anat. Rec.* **142** 41-52
- Humason G L 1965 *Animal tissue techniques* (San Fransisco and London : W H Freeman)
- Hunt T E and Hunt E A 1959 Glycogen in the adrenal gland of rats at different ages ; *Anat. Rec.* **133** 537-551
- Lillie R D 1965 *Histopathological technique and practical histochemistry* (New York : Blakiston Div., McGraw-Hill)
- McKeever S and Tomich P Q 1963 Observations on the adrenal glands of the mongoose ; *Anat. Rec.* **147** 163-170
- McManus J F A and Mowry R W 1960 *Staining methods : Histological and histochemical* (New York : Paul B. Hoeber)
- Meyers M W and Charipper H A 1956 A histological and cytological study of the adrenal gland of the golden hamster (*Cricetus auratus*) in relation to age ; *Anat. Rec.* **124** 1-25
- Pauly J E 1957 Morphological observation on the adrenal cortex of the laboratory rat ; *Endocrinology* **60** 247-264
- Pearse A G E 1968 *Histochemistry : Theoretical and applied* 3rd edn. (London : J A Churchill)
- Prasad G and Yadava R C P 1974 Histological and histochemical studies on the adrenal glands of the Indian buffalo ; *Indian J. Anim. Sci.* **44** 243-248
- Sinha D and Ghosh A 1961 Some aspects of adrenocortical cytochemistry in the domestic pigeon ; *Endokrinologie* **16** 270-280 (Vide *Excerpta Med.* **16** 742-743, 1962)
- Sohal H S and Chaturvedi R P 1962 Adrenal glands of Indian buffalo ; *J. Anat. Soc. India* **11** 46

Effect of x-rays on the somatic chromosomes of the exotic fish, *Tilapia mossambica*

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Abstract. Male and female *T. mossambica* were x-rayed with 100 r and the metaphase chromosome aberrations in their gill epithelia were studied at 13 different intervals against suitable control. The chromosomes of males appeared more radio-sensitive than those of females. Among the diploid complement of 44 chromosomes, the individual type aberrations were non-random in both sexes. The longest pair of chromosomes, taken as the marker pair, was found very highly radio-sensitive, while the remaining 21 pairs as non-markers were somewhat resistant to x-radiation when the observed and the expected numbers were subjected to statistical analysis. The break in the marker chromosome was also non-randomly distributed as the distal half had a significantly large number of breaks.

Keywords. Fish; *Tilapia mossambica*; x-irradiated chromosome aberrations; differential radio-sensitivity.

1. Introduction

In comparison to some insect and mammalian models, very limited studies on the radiation induced chromosome aberrations in fish have so far been carried out. Such studies have, however, dual importance because fish in general serve as an important biological monitor in aquatic environment for the study of radiation pollution and secondly their stock could be improved through radiation induced mutation and selection. Schroder (1973) reviewed the works on radiation induced mutations in fish while Hickling (1962) reported the genetics and hybridization effect of some fish including *Tilapia*. At the chromosomal level Hama *et al* (1976) studied the chromosome aberrations in gill epithelia of the mosquito fish, *Oryzias latipes* from 2 to 10 days after radiation, while Pechkurenkov (1976) studied the chromosome aberrations in embryonic fish induced by chronic radiations. The dose-dependent effects of x-rays on the frequency of mitosis in regenerating tail fin of *O. latipes* was studied by Hama and Egami (1977). Mong and Bena (1979) also studied the effect of x-rays on chromosomes of mud minnow using different doses. The present paper deals with the x-ray induced chromosome aberration in the fish *T. mossambica* with special reference to the study of the differential radio-sensitivity of chromosomes between males and females and between and within the chromosomes in each sex which were not studied before. *T. mossambica* has been chosen not only for its easy rearing and handling, but

also its mitotic metaphase complements containing a pair of conspicuously large chromosomes which, as markers, served better to study the problem of the intraand interchromosomal radio-sensitivity.

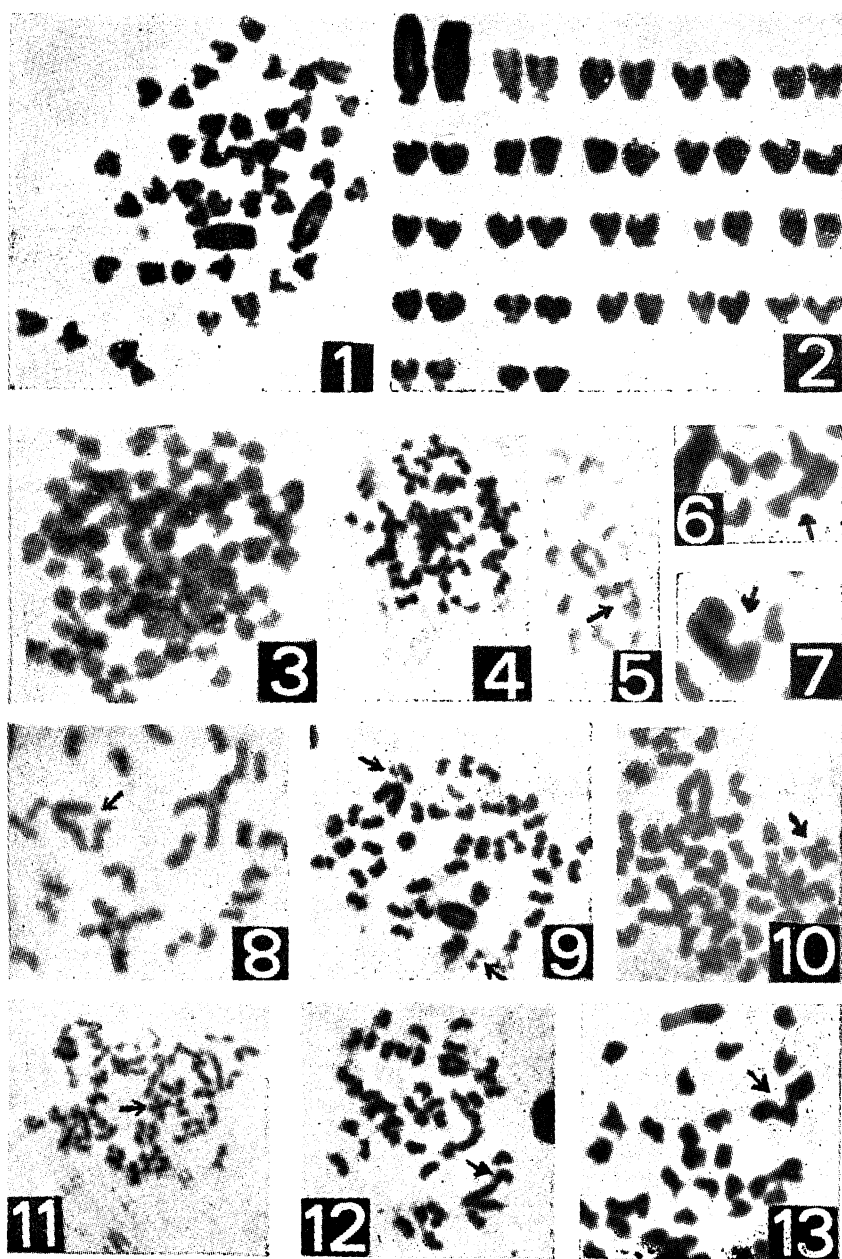
2. Material and methods

The herbivorous freshwater higher group of teleostean fish, *Tilapia mossambica* Peters (Family Cichlidae, Order Perciformes) domestic to the rivers of East coast of Africa was introduced to the Indian inland waters for its exotic habit of breeding. They breed throughout the year almost every 2 months except in winter (see Jhingram 1974). Specimens used in the present investigation were from the 4th inbred generation raised by us. Before irradiation living male and female specimens were acclimatized in the aquarium for a day or two. Immediately after taking them out of the aquarium, their body was gently rubbed once with a piece of dry cloth to remove surface water. They were then irradiated with the dose of 100 r from the x-ray machine operated at 110 kV, 4 mA with 1 mm aluminium filter emitting 2.5 r per second. After irradiation the specimens were stocked into the aquarium for fixing their gills at different intervals. As controls unirradiated specimens of the same brood were kept into another aquarium under similar laboratory conditions. An hour before the fixation time each specimen was intramuscularly injected with 0.1% colchicine solution at the rate of 2 ml per 100 gm body weight. No colchicine was injected if the fixation of the tissue was to be done within an hour after irradiation. The gills of each specimen immediately after removal were minced in 1% sodium citrate solution and the minced tissue was left into citrate solution for an hour at room temperature. The tissue was then fixed in acetic-alcohol (1:3) mixture for a brief period after removing the citrate solution by centrifugation. The fixed tissue suspension was taken on a slide and after air-drying the slide was stained with Giemsa stain at pH 7.2. The observations were made from the stained air-dried slides.

3. Observations

3.1. Control series

The diploid number of chromosomes in both the sexes of *T. mossambica* was 44, the sex chromosomes being undifferentiable cytologically (figure 1). With regard to the morphology of the chromosomes different workers (Natarajan and Subramaniam 1968; Hideo and Muramoto 1975; Prasad and Manna 1976; Manna and Som, unpublished) were not in complete agreement with one another excepting, of course on the first pair of the longest subtelocentric chromosomes, referred to here as the marker pair. The controversy was on the exact morphology of the remaining 21 pairs of non-marker chromosomes. Their relatively small size and variable length and disposition of the shorter arm caused confusion. Anyhow none of the chromosomes was of the true metacentric type which helped us to determine the cases of centric fusion leading to form metacentric chromosome in the treated material (*vide infra*). Thus, without entering into any controversy, for our present analysis we put the first longest pair into the marker group and

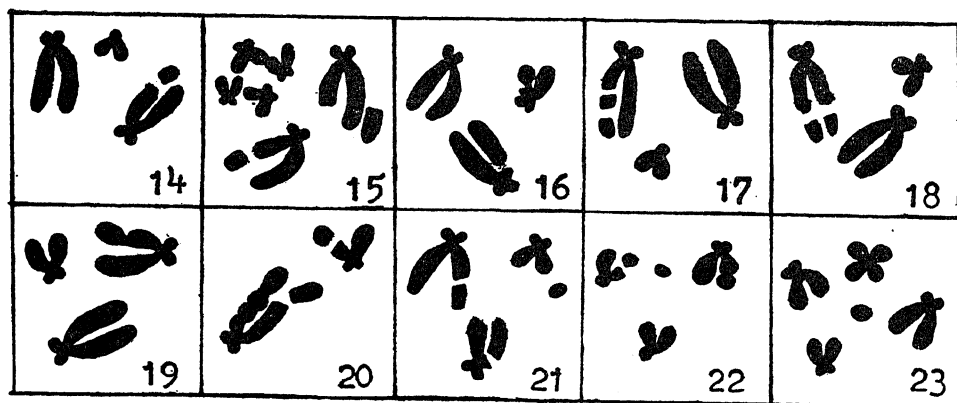


Figures 1-13. Photomicrographs, part and full metaphases. 1. A normal complement in male ($2n = 44$), 2. Male karyotype, 3. Polyploidy, 4. Stickiness, 5. A marker chromosome with a subchromatid break, 6-8. Each with a chromatid break in a marker chromosome, 9. Two isochromatid and one chromatid fragment of unknown origin, 10, 11. Each with a small metacentric chromosome formed by the centromeric fusion of two non-marker chromosomes, 12, 13. Terminal association and/or chromatid exchange between two chromosomes.

the remaining 21 smaller pairs into the non-marker group (figure 2). Since the first marker pair was about double the size of the second pair (figure 2), there was not the least difficulty in identifying the first marker pair in any plate. This marker pair formed $1/22$ part in the haploid number and approximately measured $1/10$ (average 15.0μ) of the total genome length (149.6μ). The second pair also considered as marker chromosome (Hideo and Muramoto 1975) is, however, not considered as its size difference from the 3rd pair is not very conspicuous (figure 2). In the control series out of 150 metaphases examined in each sex at each of the 13 intervals corresponding to the treated series (table 1), only 2 constrictions were encountered at 96 hr in females. Thus these two were the individual type solely found in a total of 3,900 metaphases examined in two sexes while there were metaphases with gross effect found at all intervals. The frequency in the combined data of two sexes was 5.0% in 5 min, 8.7% in 1 hr, 4.3% in 6 hr, 6.0% in 12 hr, 8.0% in 24 hr, 8.7% in 48 hr, 3.3% in 72 hr, 7.0% in 97 hr, 5.0% in 120 hr, 6.0% in 144 hr, 5.0% on 7th day, 7.7% on 10th day, 3.7% on 15th day and 6.0% as average (table 1). The gross effect was mainly due to the stickiness of chromosomes and the frequency fluctuated erratically.

3.2. X-rayed series

In comparison to the control series, the gill epithelia of the x-irradiated specimens contained various types of aberrations (figures 3-23). For the sake of convenience



Figures 14-23. Camera lucida drawings \times ca. 3,000 showing some rearranged metaphase chromosomes mostly with aberrations induced by x-rays. 14. One marker chromosome with a terminal chromatid break 15. Each marker chromosome with a chromatid break, and three non-markers with terminal association or chromatid exchange, 16. A marker chromosome with a proximal chromatid break and a non-marker chromosome with a constriction, 17. A marker chromosome with two breaks in the same chromatid, 18. A marker chromosome with isochromatid breaks, 19. A marker chromosome with a chromatid constriction, 20. A marker chromosome with beaded constrictions in one chromatid and the other with a break while a non-marker chromosome with a chromatid break, 21. A chromatid gap in a marker, a chromatid break in the first non-marker (2nd marker) chromosome and a fragment of unknown origin, 22, 23. Each with a fragment of unknown origin while one (No. 22) also contained a chromatid break in a non-marker chromosome and the other (No. 23) a centric fusion,

Table 1. The frequency of chromosome aberrations in 300 metaphases (150 each of male and female) at each interval of 100r x-irradiated *Tilapia mossambica*. Data on female are in bracket ().

Fixa time	Individual type aberrations					No meta gross effect	Total affected metaphase	% in sex combined aff. met.		% Net increase	
	Breaks	Fragm	Trans	Gaps and Cons	Total			No meta involved	Treated		Control
5 mi	13 (14)	12 (8)	- (-)	12 (9)	37 (31)	26 (18)	18 (29)	44 (47)	30.3	5.0	25.3
1 hr	13 (7)	9 (7)	1 (1)	14 (11)	37 (26)	21 (12)	22 (18)	43 (30)	24.3	8.7	15.6
6 hr	6 (4)	4 (3)	1 (2)	10 (8)	21 (17)	17 (15)	8 (3)	25 (18)	14.3	4.3	10.0
12 hr	11 (10)	7 (6)	2 (1)	6 (9)	26 (26)	19 (16)	26 (30)	45 (46)	30.3	6.0	24.3
24 hr	5 (8)	4 (1)	3 (2)	7 (4)	19 (15)	12 (9)	27 (10)	39 (19)	19.3	8.0	11.3
48 hr	10 (12)	8 (9)	1 (2)	9 (7)	28 (30)	22 (19)	25 (27)	47 (46)	31.0	8.7	22.3
72 hr	6 (9)	4 (3)	2 (2)	7 (3)	19 (17)	15 (12)	18 (26)	33 (38)	23.7	3.3	20.4
96 hr	6 (9)	7 (6)	1 (2)	8 (10)	22 (27)	13 (12)	25 (25)	38 (37)	25.0	7.0	18.0
120 hr	5 (5)	2 (2)	- (1)	6 (5)	13 (13)	9 (9)	23 (20)	32 (29)	20.3	5.0	15.3
144 hr	9 (4)	4 (3)	3 (1)	- (2)	16 (10)	13 (10)	6 (10)	19 (20)	13.0	6.0	7.0
7 day	6 (2)	1 (1)	- (-)	5 (5)	12 (8)	7 (7)	1 (1)	8 (8)	5.3	5.0	0.3
10 day	- (-)	2 (2)	- (-)	3 (3)	5 (5)	3 (3)	22 (17)	25 (20)	15.0	7.7	7.3
15 day	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	10 (9)	10 (9)	6.3	3.7	2.6
Total	90 (84)	64 (51)	14 (14)	87 (76)	255 (225)	177 (142)	231 (225)	408 (367)	19.8	6.0	13.8

polyploidy (figure 3), stickiness (figure 4), *c*-mitosis etc. were put under gross effect in which the entire chromosome complement was affected while subchromatid (figures 5, 14), chromatid (figures 6-8, 14-17, 20, 21) and isochromatid (figure 18) breaks, fragment of unknown origin (figures 9, 22, 23), translocation and fusion (figures 10-13, 15), constriction (figures 16, 19, 20), gap (figure 21) etc. were put under individual effect in which one (figures 5-8) or more (figures 14-21) chromosomes of the whole complement were involved. It appeared that the individual type aberrations were mostly of the chromatid type. If the marker chromosome was arbitrarily divided into 3 equal regions as proximal, middle and distal from the centromeric end, the chromatid breaks were somewhat localized in the distal region because out of 106 breaks in the marker pair, 15 were in the proximal, 33 in the middle and 58 in the distal region against the expected number of 35.3 breaks per region with random occurrence. The difference was statistically significant at 1% level ($\chi^2 = 26.40$, d.f. 2). Thus, broadly speaking the distal half was more radio-sensitive than the proximal half of the marker chromosomes. However, in the present material such an analysis in the non-marker chromosomes was not possible for the inherent difficulties with the morphology and size of chromosomes. Definite translocation between the marker and non-marker chromosomes except for some terminal chromatid association or exchange (figure 12) was not encountered but centric fusion between two non-marker chromosomes (figures 10, 11) was common. While scoring the data some individual type of aberrations in the non-marker group might have escaped due to the inherent observational difficulty for the small size and morphology of the chromosomes. But the frequency of such an omission, if it occurred at all, would not exceed more than 2%.

In presenting the data the different individual type aberrations were put into one category *e.g.*, subchromatid, chromatid and isochromatid breaks as breaks etc. while for the gross types all of them were put together (table 1). An analysis of the data (table 1) indicated that chromosomes in irradiated males were affected more than those in females because in the same number of 150 metaphases, the number of aberrations was higher in males at all intervals and in their total except at 5 min, and from 24 hr to 96 hr for breaks, except at 48 hr for fragment of unknown origin and except at 12 hr, 96 hr and 144 hr for gaps and constrictions.

As the translocation data were limited, we made no comment. The statistical analysis of the total data showed that the difference was below the significant level because $\chi^2 = 0.20$, d.f. 1 for breaks, $\chi^2 = 1.47$, d.f. 1 for fragment of unknown origin and $\chi^2 = 0.74$ for gaps and constrictions. In the combined data of all individual type aberrations, it was also higher in males at each interval except at 48 hr and 96 hr (table 1). In the total of all intervals, the males had 255 aberrations against 225 in females. The difference was also a little below the significant level because the χ^2 value was 1.87 with 1 d.f. Therefore, on the whole the higher frequency of individual type aberrations in males was somewhat indicative that the sex factor might have some differential radio-sensitivity, but the data needed be extended for further confirmation. That the sex factor could have differential radio-sensitivity was supported by the fact that when the number of affected metaphases which contained individual type aberrations was compared between the two sexes, it was found higher in males in 9 intervals while

it was at par with females at 120 hr, 7th day and 10th day (table 1). In the total 1950 metaphases 177 were affected in males against 142 in females. The χ^2 test gave a value of 3.84 with 1 d.f., indicating that the difference was significant at 5% level.

The number of affected metaphases with gross effects like polyploidy, stickiness etc. was not significantly different in the two sexes. It was a little higher in males at 6 out of 13 intervals and in the total (table 1). The χ^2 value was 0.08 with 1 d.f. which indicated that the difference was highly insignificant. This was expected because gross effect was mostly physiological in origin. The number of affected metaphases with individual and gross type aberrations if combined, would be higher in males in 8 out of 13 intervals and in the total (table 1). The χ^2 test showed that the value 2.17 with 1 d.f. was a little below the significant level. Thus, though the analysis of the data of aberrations and the affected metaphases did not conclusively prove, that the males and females responded differentially, there were reasonable indications beyond doubt for the same.

The individual type aberrations did not show a regular mode of incidence in both the sexes. The maximum number of the different types was mostly found in 5 min (breaks, fragments in male, total) and 1 hr (gaps and constriction) which reduced to nil on the 15th day or earlier (breaks) but the mode of decrease was very erratic as number fluctuated oddly at different intervals (table 1). The frequency of affected metaphases in male showed the same trend but in female the maximum number was found at 48 hr. The occurrence of the affected metaphases with gross effects was still more erratic as the maximum number of 27 was found at 48 hr in males and 29 in females at 5 min and the effect continued in a lower frequency in both sexes even on the 15th day (table 1). On the whole, the present data showed that the individual type of aberrations did not continue up to the 15th day while the gross type continued longer and in both cases the frequency fluctuated at different intervals (table 1). That the x-radiation induced a higher frequency of chromosome aberrations and affected more metaphases was beyond any doubt. The net increase in the individual type aberrations when the data of two sexes were combined was 22.7% at 5 min, 21.0% at 1 hr, 12.7% at 6 hr, 17.3% at 12 hr, 11.3% at 24 hr, 19.3% at 48 hr, 12.0% at 72 hr, 15.7% (2 constrictions in control) at 96 hr, 8.7% at 120 hr and 144 hr, 6.7% on 7th day, 3.3% on 10th day and nil on 15th day. The net increase in an average was 12.4%. On the other hand, the net increase in the frequency of total affected metaphases over the control was 25.3%, 15.6%, 10.0%, 24.3%, 11.3%, 22.3%, 20.4%, 18.0%, 15.3%, 7.0%, 0.3%, 7.3% and 2.6% respectively in 13 intervals and 13.8% in the average (table 1).

3.3. *Non-random distribution*

To find out if the aberrations were non-randomly distributed between the marker and non-marker chromosomes, some individual type aberrations like breaks, gaps and constrictions were quantitatively assessed at each interval from 150 metaphases examined in each sex (table 2). The other individual types like fragment of unknown origin and translocation were not considered as the chromosome involved was not known in the former type.

Table 2. Frequency distribution of some individual type aberrations between 1st 'Marker' pair and 21 pairs of non-marker chromosomes in X-irradiated male and female *Tilapia mossambica*. Data of females are in brackets ().

Fixa time	No of metaphase	Marker chromosome			Non-marker chromosome			Grand total
		Break	Gap and Cons	Total	Break	Gap and Con.	Total	
5 min	150 (150)	7 (7)	4 (2)	11 (9)	6 (7)	8 (7)	14 (14)	25 (23)
1 hr	150 (150)	5 (1)	8 (5)	13 (6)	8 (6)	6 (6)	14 (12)	27 (18)
6 hr	150 (150)	5 (3)	7 (8)	12 (11)	1 (1)	3 (-)	4 (1)	16 (12)
12 hr	150 (150)	7 (7)	6 (8)	13 (15)	4 (3)	- (1)	4 (4)	17 (19)
24 hr	150 (150)	4 (7)	4 (4)	8 (11)	1 (1)	3 (-)	4 (1)	12 (12)
48 hr	150 (150)	9 (11)	8 (2)	17 (13)	1 (1)	1 (5)	2 (6)	19 (19)
72 hr	150 (150)	5 (3)	6 (3)	11 (6)	1 (6)	1 (-)	2 (6)	13 (12)
96 hr	150 (150)	4 (5)	6 (6)	10 (11)	2 (4)	2 (4)	4 (8)	14 (19)
120 hr	150 (150)	4 (5)	4 (4)	8 (9)	1 (-)	2 (1)	3 (1)	11 (10)
144 hr	150 (150)	3 (2)	- (2)	3 (4)	6 (2)	- (-)	6 (2)	9 (6)
7 day	150 (150)	- (2)	2 (3)	2 (5)	6 (-)	3 (2)	9 (2)	11 (7)
10 day	150 (150)	- (-)	3 (1)	3 (1)	- (-)	- (2)	- (2)	3 (3)
15 day	150 (150)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
Total obs.	1950 (1950)	53 (53)	58 (48)	111 (101)	37 (31)	29 (28)	66 (59)	177 (160)
Expected per number				8 (7)			169 (153)	177 (160)
Expected per length				18 (16)			159 (144)	177 (160)

It was interesting to note that there was some difference in the data of the two sexes. In the marker chromosome no difference was seen in the total number of breaks, while it was higher by 10 in males for gaps and constrictions (table 2). In the non-marker chromosomes it was higher in males by 6 for breaks and meagrely by 1 for gaps and constrictions. Therefore, no definite claim was made as to the differential response of the two sexes, it was just to draw attention to the trend.

That the marker chromosomes in each sex were highly sensitive to x-radiation was clear when the observed and the expected values calculated according to the number of chromosomes and according to the mean length were compared. Out of the total 177 individual type aberrations in males, 111 were observed in the marker pair-against the expected number of only 8 as calculated per proportionality of number indicating thereby that the marker pair was about 14 times more susceptible to x-ray damages. The expected number was 18 if the mean length was considered. Even then the observed number was more than 6 times indicating the higher susceptibility of the marker pair. On the other hand, in the non-maker chromosomes of males, 66 aberrations were found against 169 expected, calculated per number of chromosomes and 159 calculated per length of chromo-

somes, indicating thereby that the susceptibility of the non-marker chromosomes was 2.5 times and 2.4 times less. The chi-square tests of the expected data per number and per length against the observed number showed in each case that the difference was highly significant ($P < 0.001$). Therefore, in males some individual type aberrations mentioned early were non-randomly distributed between the marker and non-marker chromosomes, the former group was highly susceptible and the latter group was somewhat resistant to the x-ray damages.

In females like males the marker chromosomes were also found to be highly radio-sensitive, while the non-marker ones were somewhat less susceptible. Out of the total 160 individual type aberrations analysed, 101 were observed in the marker pair against the expected number of 7 as calculated per proportionality of number and 16 as calculated per length of chromosomes (table 2). Thus, like in males, in females also the marker chromosomes were 14 times more susceptible according to the number and over 6 times susceptible according to the length of chromosomes. On the other hand, in the non-marker chromosomes the observed number of individual type aberrations was 59 as against the expected number of 153 calculated per proportionality of number and 144 calculated per proportionality of length of the non-marker chromosomes, indicating that they too, like marker chromosomes, were 2.5 times less vulnerable according to the number and 2.4 times less vulnerable according to the length of chromosomes. The chi-square tests of the observed number and the expected number calculated per number and the expected number calculated per length of chromosomes showed that the difference in both the cases was very high ($P < 0.001$). Therefore, just like males, the two groups of chromosomes showed the same type of response to x-rays, the marker chromosomes were highly susceptible, while the non-markers were somewhat less responsive.

Since the data of each sex showed differential radio-sensitivity, it was expected naturally that in the combined data of the two sexes the same manifestation would be shown. Thus, out of the total 337 aberrations, 212 were observed in the marker pair against the expected numbers of 15 and 34 calculated per number and length of chromosomes respectively which also showed 14 times and 6 times more susceptibility of the marker chromosomes (table 2). In the non-marker chromosomes 125 aberrations were observed as against the expected number of 322 and 303 calculated per number and per length of chromosomes respectively which also showed that the non-marker chromosomes were 2.5 times and 2.4 times less vulnerable to x-ray damages. The chi-square test of the observed number and the expected numbers calculated in two different ways showed in each case that the difference was highly significant ($P < 0.001$).

4. Discussion

Most of the effects of ionizing radiations on chromosomes of fish reported by different investigators did not elaborate on the aberration types. The qualitative aspect of the present study revealed that the aberrations were of a similar nature as found in the somatic chromosomes of some classic material induced by radiations. But as the chromosomes of fish were not cytologically ideal, all types could not be studied in every detail. The individual type aberrations

could only be studied more elaborately in the marker chromosomes. The quantitative study of the chromosome aberrations in *T. mossambica* at different intervals showed that the effect lingered for a long time. There was not much indication of the cell lethality caused by the dose of 100r. The individual types continued mostly up to the 10th day, while the gross type did so till the end of the fixation intervals. Anyhow the persistence of mainly the chromatid type aberration as long as 10 days after irradiation deserved some consideration. Though the timing of the cell cycle in *T. mossambica* has not been worked out, yet within 10 days some cells must have completed the cycle unless their further division was inhibited. The prevalent occurrence of the chromatid type aberrations at late intervals indicated the possibility. Further, the chromatid type break has been supposed to be induced by the radiation acting on the post-synthetic period of DNA or else after the replication of chromosome, the reason for which could not be suggested. It has been a matter of common experience that the chromosome aberrations induced by odd chemical (Kihlman 1966; Manna 1971, 1975, 1978) and living mutagens (Manna 1980) were mainly of chromatid type. The same type of chromosome response of having mainly chromatid type aberrations to ionizing radiations like other chemically induced ones might lead us to think that the post-synthetic period, in general, was most sensitive for mutagenic damage to chromosomes. Since the aberrations were found within 5 min after radiation it was all the more suggestive that the chromosome nearing metaphase was more vulnerable to x-ray lesion. The occurrence of more or less the same chromatid type aberrations from the beginning to almost the end of fixation interval would further lead us to suspect if the chromosomes approaching metaphase were the vulnerable stage. This was suggested to explain the chromosome aberrations induced by odd mutagens in mice (Manna 1971, 1975). The present study indicated the differential radio-sensitivity of chromosomes and metaphase nuclei of males and females irradiated with x-ray. In the past various parameters were used to test the differential radio-sensitivity in different materials (Evans 1962; Sparrow 1962; Manna and Mazumder 1968) while the testing of the differential radio-sensitivity of chromosomes in the two sexes in fish has not been carried out. The present data need be extended to confirm because there were some lecurae in the data.

The analysis of the data of the region-wise distribution of chromatid breaks in the marker chromosomes of *T. mossambica* revealed that the distal region or more broadly the distal half was more vulnerable to x-ray damages. More or less the same trend was shown by the chromosomes of mice treated with physical, chemical and living mutagens (Manna 1971, 1975, 1978, 1980) for which it was suggested by Manna (1975, 1978) that there could be some inherent weaker region in chromosomes. The same might be the reason for the somewhat localized break found in the marker chromosome of *T. mossambica*. The other possibility of having localized breaks by radiation was the differential restitution as suggested to explain the localized breaks in the X chromosome of irradiated grasshopper (Manna and Mazumder 1962).

Interchromosomal radiation damages by x-ray have been studied in different animals. The differential radio-sensitivity between chromosomes of the same species was seen in the Syrian hamster (Manna and Dey 1981), grasshopper (Manna and Mazumder 1962, 1968) and Heteroptera (Manna and Dey 1978,

1980). In the above cases the differential radio-sensitivity was shown between the sex chromosome and autosome of the species concerned, while in the present study on *Tilapia*, it was found between two groups of autosome. It was claimed that the radiation injury was directly proportional to the chromosome volume (Marshak 1937), length etc. but it was not found in other material (Manna and Mazumder 1968). It was also not supported from the present data because the marker chromosomes had more breaks than the expected number calculated proportional to the length of the chromosomes. The present study, therefore, revealed some interesting results on the x-ray induced chromosome aberrations in *T. mossambica*. Further studies are in progress.

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References

- Evans H J 1962 *Chromosome aberrations induced by ionizing radiations*. In : *Int. Rev. Cytol.* (eds.) G H Bourne and J F Danielli (New York : Academic Press) 13 221-308
- Hama A and Egami N 1977 The dose-rate effect of gamma radiation on the initiation of mitosis in the regenerating tail fin of the fish *Oryzias latipes*, *J. Fac. Sci. Univ. Tokyo*, Sec. IV, 14 47-60
- Hama A, Egami N, Arai R and Shiotsuki K 1976 Note on chromosome abnormalities found in irradiated *Oryzias latipes*, *J. Fac. Sci., Univ. Tokyo*, Sec. IV, 13 405-408
- Hickling C F 1962 In *Fish culture* (London : Faber and Faber) pp. 245-259
- Hideo F and Muramoto J I 1975 Somatic and meiotic chromosomes of *Tilapia mossambica* Peters. *Chromosome Information Service, Japan* 18 4-6
- Jhingram V J 1974 *Fish and fisheries of India* (Delhi : Hindusthan Publishing Corporation)
- Kihlman B A 1966 *Actions of chemicals on dividing cells* (New Jersey : Prentice Hall)
- Manna G K 1971 Bone marrow chromosome aberrations in mice induced by physical, chemical and living mutagens, *Proc. 1st All India Cong. Cytol. Genet.* (Chandigarh), *J. Cytol. Genet. (Cong. Suppl.)* pp. 144-150
- Manna G K 1975 Induced breakage in chromosomes—mechanical and/or chemical. *Proc. Symposium on structural and functional aspects of chromosomes* (Bombay, BARC) March 25-26, pp. 194-204
- Manna G K 1978 Nonrandom aberrations and their implications on the possible existence of weaker regions in chromosomes. *Proc. 3rd All India Cong. Cytol. and Genet.* (Hissar) In : *Perspective of Cytology and Genetics* (eds.) G K Manna and U Sinha (Delhi : Hindasia Publisher) 3 221-233
- Manna G K 1980 The living mutagens. In : *Golden Jubilee Commemoration Volume of National Academy of Sciences, India* (Allahabad) (ed.) U S Srivastava (Calcutta : Naya Prakash) pp. 573-606
- Manna G K and Dey S K 1978 X-ray induced chiasma-like configuration between sex chromosomes of the pyrrhocorid bug, *Physopelta schlanbuschi*. *Proc. 3rd All India Cong. Cytol. Genet.* (Hissar), In : *Perspective of Cytology and Genetics* (eds.) G K Manna and U Sinha (Delhi : Hindasia Publisher) 3 367-672
- Manna G K and Dey S K 1980 Preferential sex chromosomal aberrations induced by X-rays and their alterations by penicillin in male heteropteran bug, *Physopelta schlanbuschi*. *Symp. on Chromosome Methodological approaches in cytogenetics* (In *Cytogenetics in India* (eds.) R P Roy and U Sinha (Delhi : Hindasia Publisher) In press

- Manna G K and Dey S K 1981 Differential X-ray sensitivity of Syrian hamster chromosome, *Nucleus* **24** 48-56
- Manna G K and Mizumder S C 1962 The grasshopper X-chromosome as an indicator for X-ray induced chromosome breakage, *Proc. Zool. Soc. (Calcutta)* **15** 103-110
- Manna G K and Mazumder S C 1968 Induced X-chromosomal aberrations in the study of intra- and interspecific radiosensitivity of grasshoppers. In : *Seminar on chromosomes*, *Nucleus* (ed.) A K Sharma (Calcutta) pp. 197-207
- Manna G K and Som R C Unpublished
- Marshak A 1937 The effect of X-rays on chromosomes in mitosis, *Proc. Natl. Acad. Sci. U.S.A.* **23** 362-369
- Mong S J and Bena T M 1979 The effects of increasing dosages of X-radiation on the chromosomes of central mud minnow ; *J. Fish Biol.* **14** 523-527
- Natarajan R and Subramanyam K 1968 A preliminary study on the chromosomes of *Tilapia mossambica* (Peters) ; *Curr. Sci.* **37** 362-363
- Pechkurenkov V L 1976 Formation of chromosomal aberrations in fish embryos induced by chronic radiation ; *Radiobiologiya* **16** 842-846
- Prasad R and Manna G K 1976 Chromosomes of fishes, *T. mossambica* and *N. notopterus* ; *Chromosome Information Service Japan* No. **21** 11-13
- Schroder J H 1973 Teleosts as a tool in mutation research. In *Genetics and Mutagenesis in Fishes* (ed.) J H Schroder (New York : Springer-Verlag) pp. 91-98
- Sharma A K and Chatterjee A 1962 Chromosome size as a factor on radio-sensitivity ; *Nucleus* **5** 67-74
- Sparrow A H 1962 The role of nucleus in determining radio-sensitivity ; *Brookhaven Lecture Series* No. 17.

Histochemical changes in *Setaria cervi* caused by certain anthelmintics

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Abstract. The present study deals with the preliminary *in vivo* screening of suramin and levamisole in rat-*Setaria cervi* system with special reference to the histochemical changes in the adult worms caused by the drugs. Levamisole proved to be highly effective as a micro- and macro- filaricidal agent. It also appears to be interfering with the normal activity of alkaline phosphatase and glycogen of the adult worms with no apparent effect on its protein content. The drug also causes irreversible paralysis in adult worms. Suramin, though an active pharmacological agent, proved to be completely ineffective on microfilariae as well as on adult worms of *Setaria cervi*. Consequently, no notable alterations in the histochemistry of the parasite following suramin treatment were observed.

Keywords. White rats ; *Setaria cervi* ; histochemical observations.

1. Introduction

Numerous anthelmintics have been tried on nematode parasites in experimental studies and their efficacy has been established; but their mode of action on the worms and the consequent biochemical or histochemical alterations brought about by the drugs are least understood. Levamisole and suramin are known potent anthelmintics. Levamisole is the newly-discovered highly potent broad spectrum anthelmintic effective on a variety of nematodes. But the mode of action of these drugs on the biochemistry or histochemistry of the parasite is not fully known. The present study deals with the preliminary screening of suramin and levamisole in rat-*Setaria cervi* system with special reference to the histochemical alterations in the adult worms caused by the drugs.

2. Materials and methods

About 20 laboratory bred white rats almost of the same age group and weight were used in the present experiment. Adult worms (*Setaria cervi*), collected from the peritoneal cavity of freshly slaughtered buffaloes, were implanted surgically into the peritoneal cavity of white rats according to the method described by Baqui

and Ansari (1975). Each rat received five adult worms of both sexes. Infected rats were divided into two groups: one for the suramin and the other for levamisole treatment. The drugs were given to the microfilaria-positive rats after a week of initial infection at the higher tolerant dose determined earlier. Levamisole and suramin were administered orally and subcutaneously at 20 mg/kg/day and 9 mg/kg/day respectively. Administration of the drugs and microfilarial count were made for 5-10 consecutive days, thereafter the treated rats of both groups were autopsied to observe the condition of the worms and the apparent effect of the drugs on the worms.

Untreated normal worms (control) and those recovered from treated autopsied rats were fixed in Carnoy's fluid and cold acetone for histochemical observations of protein, glycogen and alkaline phosphatase activities. Fixed materials were cleared in benzene and paraffin blocks were made. Protein and glycogen were localized by Mercury-bromophenol blue and carmine stain methods respectively as suggested by Pearse (1960). Alkaline phosphatase was estimated by calcium cobalt technique as described by Gomori (1952).

3. Results

It was observed that all the rats treated with levamisole for 5 consecutive days cleared of microfilariae (response 100%) from peripheral blood circulation (table 1). Microfilarial density continued to drop after the administration of the very first dose of the drug. Further, rats autopsied after the disappearance of microfilariae on the 15th day of infection showed only 20% recovery of live active adult worms (table 1). The remaining worms were either completely exhausted or degenerate. Some of the worms were completely well organized in their architecture but remained immobile and inactive even after transfer to the normal saline showing the sign of doubtful viability. Such worms were also counted as dead. Posterior $\frac{1}{4}$ part of some live adult worms (male and female both) was found to be completely shrunk and contracted which remained unchanged even after transferring into the normal saline indicating the paralysing action of the drug.

Histochemical observations of the levamisole-treated worms revealed that protein content of cuticle, body muscles, boundary walls of ovary, uterus, microfilariae and developing embryos remained unchanged as compared to that of normal control. However, a heavy concentration of alkaline phosphatase found in subcuticle body muscles, lateral cords, embryos and microfilariae in control worms (figure 1) was noted to have considerably decreased in treated worms (figure 2). Similarly, glycogen content appreciably localized in muscles, boundary walls of uterus and developing embryos of control (figure 3) was also found to have relatively decreased in treated worms (figure 4).

Another drug, suramin, was found to be completely ineffective on microfilariae as well as adult worms of *S. cervi*. Some of the rats (50%) treated for 10 consecutive days did not show any sign of effectiveness on circulating microfilariae, consequently microfilarial density continued to increase in the peripheral blood circulation (table 1). Treated rats autopsied at 5 and 10 days intervals did not show any apparent macrofilaricidal effect either. Live worms recovered on autopsy ranged from 40-60%. Further, no notable changes, in all the three biochemical

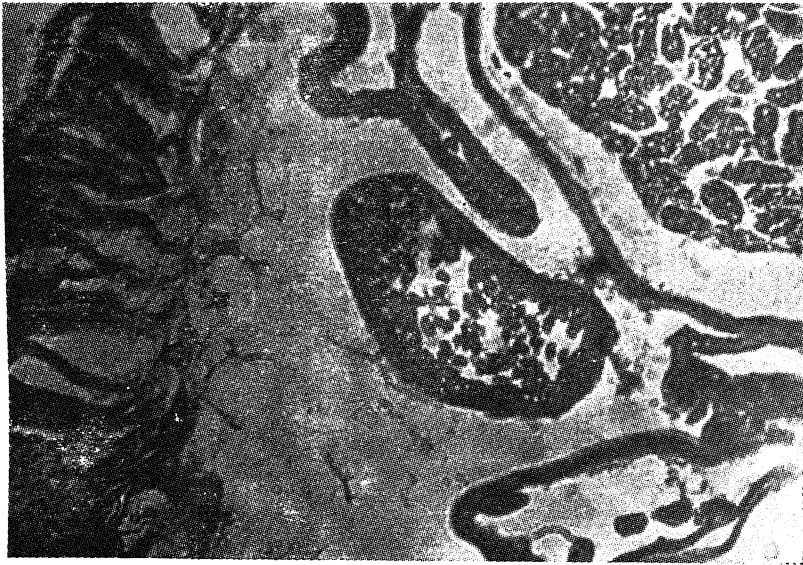
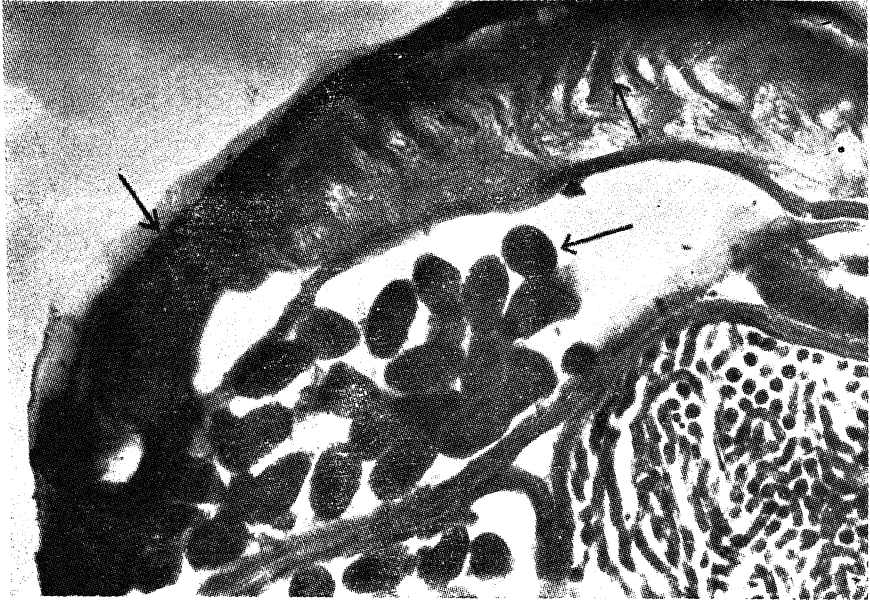


Figure 1. Alkaline phosphatase activity in the control worm.

Figure 2. Alkaline phosphatase activity in the levamisole-treated worm.

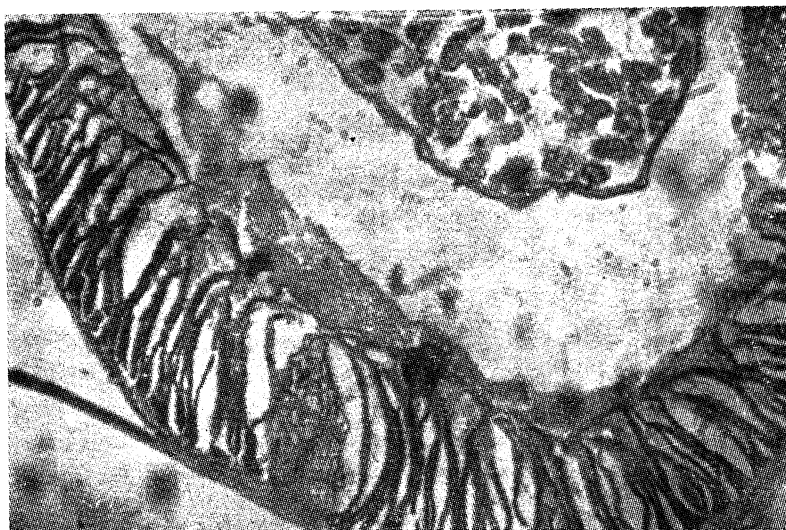
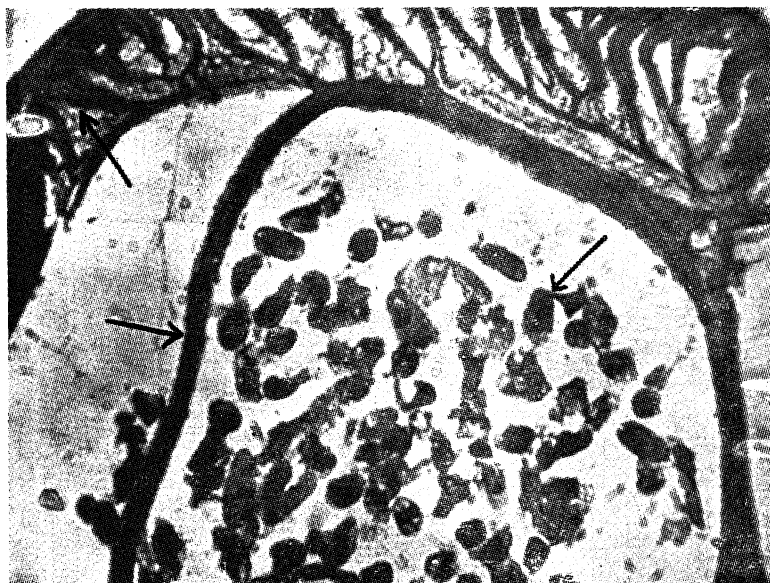


Figure 3. Glycogen localization in the control worm.

Figure 4. Glycogen localization in the levamisole-treated worm.

Table 1. The effect of certain anthelmintics on microfilariae and adult worms of *Setaria cervi*.

Drug	Dosage (route)	Mean microfilarial density/ mm ³ of blood		Duration of medication (in days)	% Recovery of live adult worms on an autopsy	Remarks
		Before treatment	After treatment			
1. Levamisole	20 mg/kg (oral)	4.5	0	5	20	Live worms active and motile; Posterior 1/4 part of some worms (20%) contracted which remained unchanged even after transfer to the normal saline. Dead worms either exhausted or completely well organized in architecture but of doubtful viability.
2. Suramin	9.0 mg/kg (subcutaneous)	3.0	12-16.4	5-10	40-60	Drug ineffective on adult worms and microfilariae. Recovered worms active and motile. Dead and exhausted worms at this stage of infection could be accounted for the natural degeneration rather than the effectiveness of the drug.

constituents, i.e., protein, glycogen and alkaline phosphatase, were recorded histochemically as compared to the control.

4. Discussion

Levamisole, a broad spectrum anthelmintic, has been found to be highly effective on microfilariae as well as adult *S. cervi* worms like its dextro-isomer, tetramisole as earlier reported by Baqui and Ansari (1976). Complete disappearance of circulating microfilariae following a 5-day treatment with levamisole and low percentage of recovery of live adult worms on autopsy are indicative of the fact that the drug contains micro—as well as macrofilaricidal property against *S. cervi*. As earlier observed, the transplanted worms normally survive in the peritoneal cavity of white rats for 4–6 weeks (Baqui and Ansari 1975). Hence, disintegration of the worms at this early stage of infection could be solely attributed to the effects of the drug.

Studies regarding the histochemical changes in the nematodes following anthelmintic treatment are scanty. However, there are a few reports on the biochemical changes of the worms brought about by certain drugs. Van den Bossche and Janssen (1969), Van den Bossche (1972), Malkin and Camacho (1972) and Prichard (1973) have reported that fumarate reductase activity is considerably inhibited in *Haemonchus contortus* and *Ascaridia galli* following treatment with tetramisole, levamisole and thiabendazole. Tetramisole also inhibits the cholinesterase, aldolase and acid phosphatase of *Ascaridia galli* (Vertinskaya *et al* 1972; Chakraborty *et al* 1976). Piperazine has been reported to decrease glycogen value in *Ascaris lumbricoides* tissues (Abdulazizov 1975; Bogoyavlenski *et al* 1975) and histamine content in *Ascaris suum* (Phillips *et al* 1976).

The present study supports the above observations. Levamisole has shown pronounced effects on adult worms which are characterized by death or irreversible paralysis of the worms. Suramin, though an effective drug in other filarial nematodes such as *Onchocerca* and *Dipetalonema* (Burch 1955; Gayral and Pommies 1976) proved to be completely ineffective on *S. cervi*. Hence no notable alterations in the histochemistry of the worms were observed. However, it has been reported that suramin inhibits strongly, *in vitro*, a variety of enzyme system of trypanosomes (Von Brand 1966).

Levamisole appears to be interfering with the carbohydrate metabolism especially with the absorption of carbohydrates and their intracellular utilization. As a result glycogen value is diminished in different organs. According to Von Brand (1966) inhibition of glucose absorption results in decrease in the concentration of energy-rich phosphate bond; finally the energy required for survival becomes inadequate and the parasite dies.

The drug also in some manner, inhibits the normal alkaline phosphatase activity of the worms as a result of which considerable decrease in its concentration in various organs is observed. The protein value remains unchanged in treated worms. There is very little information available, concerning nematode parasites, as to whether anthelmintics attack the parasite proteins or interfere with some phase of its nitrogen metabolism. Levamisole appears to have a paralysing action on adult worms and probably acts as a neuromuscular blocking agent like its

dextro-isomer, tetramisole (Gaitonde 1971). The sustained contracture of the somatic muscles of *S. cervi* results in the irreversible paralysis of the worm—a condition similarly reported in another filarial worm, *Breintia sergenti* and *Ascaris* following *in vitro* treatment with levamisole (Natarajan *et al* 1974; Van den Bossche 1972).

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References

- Abdulazizov A I 1975 Histological changes in *Ascaris lumbricoides* caused by some anthelmintics ; *Tr. Mosk. Med. Inst.* **84** 14–17
- Baqui A and Ansari J A 1975 Electrophoretic patterns of white rat sera infected with *Setaria cervi* ; *Indian J. Helminthol.* **28** 106–115
- Baqui A and Ansari J A 1976 Comparative studies on the chemotherapy of experimental *Setaria cervi* infection ; *Jpn. J. Parasitol.* **25** 409–414
- Bogoyavlenski Yu K, Abdulazizov A I and Onushko N V 1975 Changes in the fine structure of the female reproductive system of *A. lumbricoides* caused by some anthelmintics ; *Tr. Mosk. Med. Inst.* **84** 45–48
- Burch T A 1955 Treatment of wucheriasis and onchocerciasis with suramin sodium ; *Am. J. Trop. Med. Hyg.* **4** 332–333
- Chakraborty A K, Mehta R K and Dutta I C 1976 Effect of tetramisole upon certain biochemical constituents of *Ascaridia galli* ; *Indian J. Exp. Biol.* **14** 585–587
- Gaitonde B B 1971 Pharmacological review of anthelmintics ; In *Soil transmitted nematodes and tetramisole*, Ethnor Ltd. (Proceeding of the symposium) April 9 and 10
- Gayral P and Pommies M 1976 Preliminary results on the use of a new rodent filaria, *Dipetalonema dissetae* in the evaluation of filaricides ; *C.R. Hebd. Seances. Acad. Sci. Paris.* **283** 861–864
- Gomori G 1952 *Microscopic histochemistry: Principles and practice* (Chicago: University Chicago Press)
- Malkin M F and Camacho R M 1972 The effect of thiabendazole on fumarate reductase from thiabendazole-sensitive and resistant *Haemonchus contortus* ; *J. Parasitol.* **58** 845–846
- Natarajan P N, Zaman V and Yeoh T S 1974 *In vitro* activity of levamisole on the infective larvae, microfilariae and adult worms of *Breintia sergenti* ; *Int. J. Parasitol.* **4** 207–210
- Pearse A G E 1960 *Histochemistry: Theoretical and applied*, 2nd edition (London: J and A Churchill)
- Phillips J L, Sturman G and West G B 1976 The interaction between anthelmintic drug and histamine in *A. suum* ; *Br. J. Pharmacol.* **57** 417–420
- Prichard R K 1973 The fumarate reductase reaction of *Haemonchus contortus* and the mode of action of some anthelmintics ; *Int. J. Parasitol.* **3** 409–417
- Van den Bossche H and Janssen P A J 1969 The biochemical mechanism of action of the antinematodal drug tetramisole ; *Biochem. Pharmacol.* **18** 35–42
- Van den Bossche H (ed.) 1972 Biochemical effects of tetramisole ; In *Comparative Biochemistry of Parasites* (New York and London: Academic Press)
- Vertinskaya M K, Govorova S V and Polyakova O I 1972 The inhibition of *Ascaridia galli* enzymes by tetramisole ; *Byull. Vses. Inst. Gelmintol. K I Skryabina* **7** 9–12
- Von Brand T 1966 *Biochemistry of parasites* (London: Academic Press)



Effect of salinity on the survival and growth of *Chanda* (= *Ambassis*) *gymnocephalus* (Lac.) fry (Pisces; Centropomidae)

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Abstract. The survival and growth of *Chanda* (= *Ambassis*) *gymnocephalus* (Lac.) fry (8.8 ± 0.2 mm) collected from Murukumpuzha Lake (9.34‰) for a ninety day period in different salinity grades were studied. A faster rate of growth is exhibited by the fish in the highest salinity grades (22.41 and 28.51‰), even though during the first month, growth and health was apparently better in the lower salinity grades (4.11, 10.21 and 16.31‰). Assimilation efficiency also showed a similar gross picture. Thus in *C. gymnocephalus*, an euryhaline species, the fry show preferred salinity gradients for optimum growth within the fluctuating salinity regime at a stable temperature ($26 \pm 2^\circ\text{C}$) and hence may make salinity bound emigrations with growth.

Keywords. Salinity; growth efficiency; assimilation efficiency; satiation; *Chanda gymnocephalus*.

1. Introduction

Chanda gymnocephalus is an euryhaline glassy perchlet inhabiting the coastal waters and the estuarine and brackish water tracts of Kerala. A shoal of fry (8.8 ± 0.2 mm) was collected from the shallow protected region of the Murukumpuzha lake (9.34‰) about 2 to 3 km away from the Perumathura bar-mouth (pozhi) which was open. According to Nair (1957) "*Ambassis gymnocephalus* spawns in coastal waters near the bar-mouth. Large quantities of the pelagic eggs spawned in the vicinity of the bar-mouth are passively carried into the lake by the strong tidal currents. Reaching the main body of the lake, these eggs and larvae drift into the shallow protected regions." In order to understand the salinity preferences for growth and the salinity bound movements of the fry and early juveniles, the salinity tolerance and its effect on the growth pattern of the fry for a three month period under laboratory conditions were studied. Salinity preferences in emigratory movements of the presmolt coho salmon have been studied by Garrison (1965) in natural waters and by Otto and McInerney (1970) and Otto (1971) under laboratory conditions. There is also considerable field information (Canagaratnam 1959, 1966; Gunther 1961; Holliday 1971) and experimental evidence (Gibson and

Hirst 1955 ; Kinne 1960 ; Holliday 1971 ; Weatherly 1972) suggesting that growth and size of euryhaline fish are influenced by salinity.

2. Materials and methods

In about four to five weeks after hatching, the larvae reach a length of 8 mm (Nair 1957). Thus the fry were about 1-1.5 months old at the start of the experiment. Immediately on arrival at the laboratory, the fry were transferred into a large cement tank (4' x 3' x 3') containing water of 10.21‰ at $26 \pm 2^\circ\text{C}$ for two days for acclimation. The salinity tolerance of the fry for a 48 hr period was studied in seven different grades (0.96, 4.11, 10.21, 16.31, 22.41, 28.51 and 31.56‰). At 31.56‰ (seawater) and 0.96‰ (wellwater) there was 70% and 30% mortality in 48 hr while there was no mortality in other grades. Therefore only the five salinity ranges (4.11 to 28.51‰) were chosen for the growth studies.

After the tolerance tests the shoal in the cement tank were divided into batches of 100 each and reared in five aquarium tanks (60 cm x 30 cm x 30 cm) in five different grades, while about 200 specimens were kept in the cement tank as control at 10.21‰. Once in the experimental tanks they were allowed a 12-18 hr period to acclimate. There was no mortality during this period. Feeding began the next morning and the fish were fed to satiation twice a day. For the first twenty days they were fed on *Artemia* nauplii and thereafter, till the end of the experiment on chopped tubifex worms (*Tubifex tubifex*).

At the end of every ten days (11, 21, 31, ..., 91) five fish were collected at random (retarded individuals were discarded) and sacrificed for length and weight measurements and the average taken for each grade. Length measurements were made using a micrometer (up to 1/100th of a mm) and weight using an electric monopan balance (up to 1/100th of a mg).

At the end of every ten days (15, 25, 35, ..., 85) the satiation amount, the amount of faecal matter excreted (in 24 hr) and the total weight of fish in each salinity grade were also measured for the rough estimation of digestibility (= digestion coefficient) as given by Kapoor *et al* (1975) and dealt with in the present study as 'assimilation efficiency', and for the estimation of growth efficiency for each ten-day period.

$$\text{Assimilation efficiency} = \frac{A_{Ia} \times 100}{A_{Ie}},$$

(Digestibility)

where $A_{Ie} = A_{Ia} - A_{Im}$.

$$\text{Growth efficiency}_{10 \text{ days}} = \frac{A_{Ia} \times G_{10}}{A_{Ie}}$$

where A_{Ia} = amount of food eaten, A_{Im} = amount of faecal matter excreted, A_{Ie} = amount of food assimilated, G_{10} = growth in weight during the corresponding ten-day period.

The different salinity ranges were prepared by diluting seawater with wellwater making 10, 30, 50, 70 and 90% seawater and were maintained throughout the

experimental period at $\pm 1\%$. Salinities were determined using a salinometer. All experimental tanks were maintained at a water temperature of $26 \pm 2^\circ \text{C}$ and the oxygen kept at air saturation level using aerators every alternate day. The excess of food was removed within two hours of feeding. To inhibit the accumulation of metabolites and bacterial growth, water was changed every fifth day.

The salinity tolerance (48 hr) of a new stock of early juveniles (22–27 mm) collected from near the bar-mouth (pozhi) was also studied.

3. Results

The cumulative growth in length and weight are plotted against time (days) for the different salinities and the corresponding regression equations are given in figures 1 and 2. The regression equations are calculated using the least square method of analysis. The correlation coefficient values for cumulative growth in length and weight for the different salinities are given in table 1. All the values are significant at the 1% level. A faster rate of growth is exhibited by the fish in the highest salinities (22.41 and 28.51‰), even though during the first month growth and health were apparently better in lower salinities (4.11, 10.21 and 16.31‰).

Estimates of assimilation efficiency and growth efficiency are given in tables 2 and 3. Changes in growth pattern are clearly reflected in the growth efficiency changes with time (figure 3) and the corresponding regression equations are given.

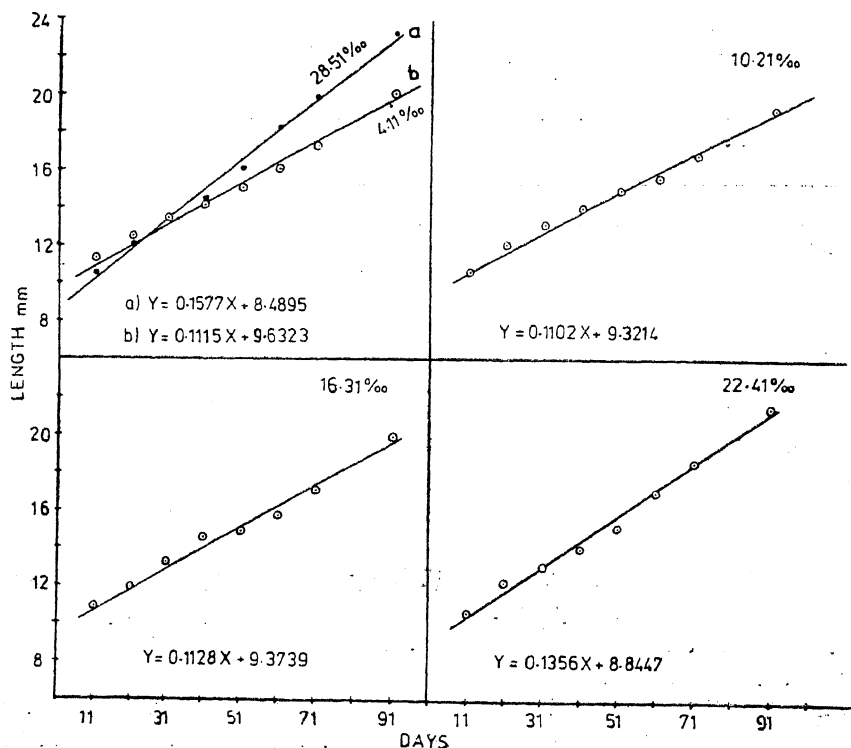


Figure 1. Regression lines and equations for growth in length in different salinities.

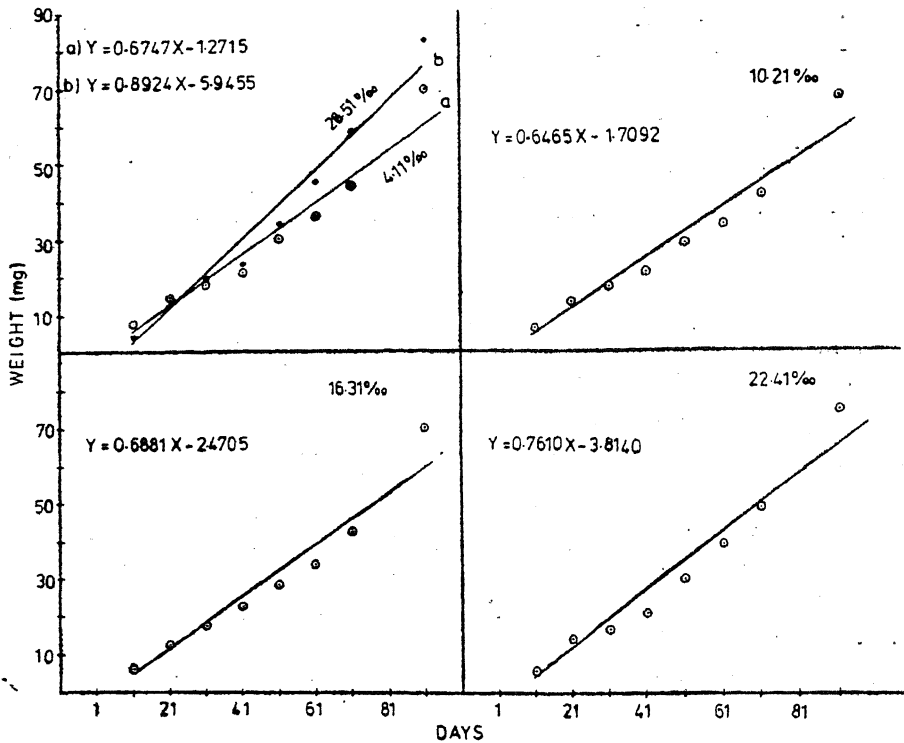


Figure 2. Regression lines and equations for growth in weight in different salinities.

Table 1. The correlation coefficient values for cumulative growth in length and weight.

Salinity (‰)	Correlation coefficient values	
	Growth in length	Growth in weight
1. 4.11	0.9925	0.9716
2. 10.21	0.9955	0.9725
3. 16.31	0.9931	0.9695
4. 22.41	0.9957	0.9735
5. 28.51	0.9974	0.9765

In the initial stages better assimilation efficiency is shown by the lower salinities, but with growth higher salinity grades show greater efficiency as may be seen in figure 4. The estimates of the satiation amount, in percentage of fish wet weight, are presented in table 4.

In the control (10.21‰) the fish weight and length were 67.4 mg and 20.1 mm at the end of the experiment (66.2 mg and 19.44 mm for the experiment 10.21‰) showing that their growth was in no way inhibited.

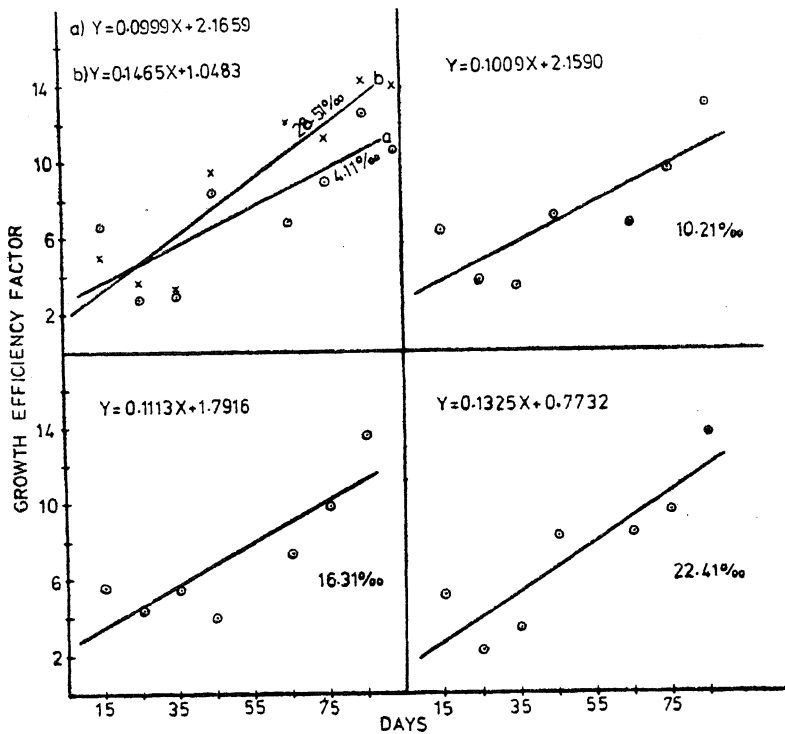


Figure 3. Changes in growth efficiency factor with time in different salinities.

The salinity tolerance range of the early juveniles was between 5.5‰ and 33.60‰ (figure 5).

4. Discussion

In order to analyse and understand growth phenomena, it is convenient to consider short growth periods or stanzas for arbitrarily defined time periods (Webb 1978).

For the initial three ten-day periods (one month), growth was inhibited in the two higher salinities of 22.41 and 28.51‰, whereas the three lower salinities had little effect on the growth, the fish showing uniform growth. Of primary interest in the series of experiments is the gradual maximisation of growth in the two higher salinities (22-29‰) and the gradual decline in the rate of growth for fish maintained at the two lower salinity grades (4-17‰). A precipitous decline in the rate of growth and growth efficiency at all salinity grades occurred during the 21-41 day period even though the assimilation efficiency was not affected. This may be due to the change in food from *Artemia* nauplii to chopped tubifex worms, since after this period growth rates picked up fast and then showed steady increase in all salinities especially in the two higher grades.

Corresponding to changes in growth rates, the growth efficiency in different salinities showed similar fluctuations (figure 3). In the initial stages, higher growth efficiency was shown by fish in the three lower salinities. Even though food

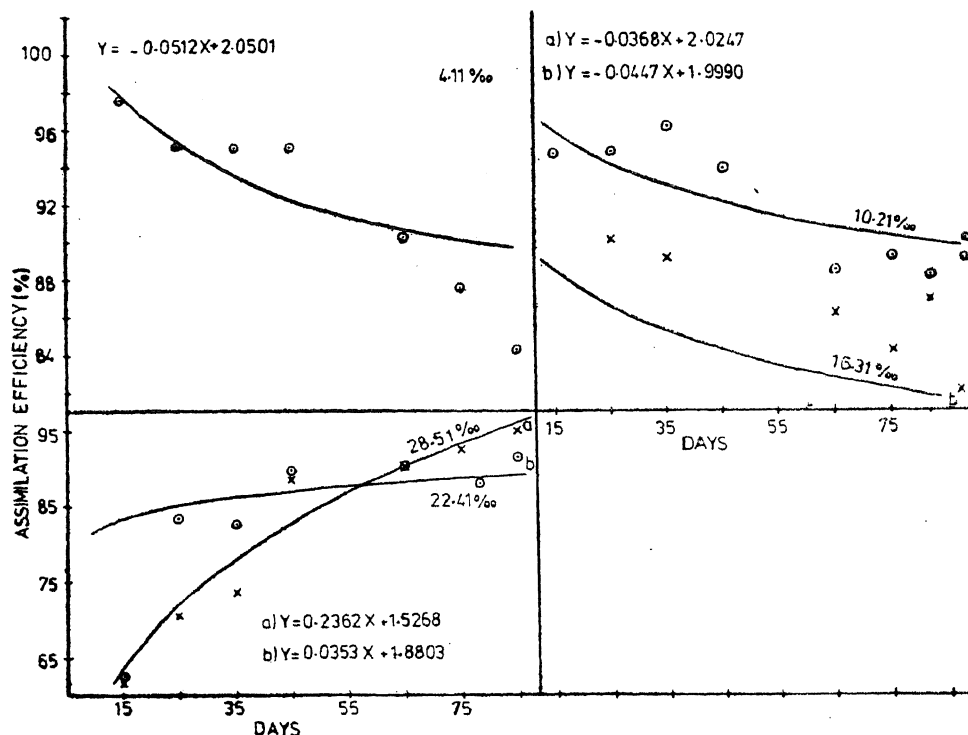


Figure 4. Changes in assimilation efficiency with growth in different salinities.

consumption was high in the higher grades at this time, the assimilation efficiency was as low as 62.5 % (22.41‰) and 61.3 % (28.51‰) whereas it was as high as 97.61 % (4.11‰) ; 94.66 % (10.21‰) and 87.99 % (16.31‰) during the 11-21 day period. But slowly the assimilation efficiency picked up in the higher salinities while the lower grades showed marginal decrease with time as is clearly brought out in figure 4. According to Paloheimo and Dickie (1966a,b) and Warren and Davis (1967) salinity may affect growth through its influence on food conversion efficiency and activity, which are important components of the bioenergetic budget of fishes, as is seen in the present study also. Also according to Webb (1978), "In general total food intake is greatest and metabolism smallest under least stress so that growth is then maximal", as is seen in the lower salinities during the initial stages and in the higher grades with acclimation and passage of time.

Another interesting aspect was the schooling behaviour of the fry. In the higher salinities during the initial stages of the experiment the fish were scattered as individuals but with passage of time formed loose-knit shoals and by the 41-51 day period were shoaling well and behaving as a unit. In the lower salinity grades the fry were shoaling well from the initial period of introduction but tended to scatter in the later stages. From the fluctuations in the assimilation efficiency it may be noted that schooling tend to reduce metabolism. Parker (1973) has made similar observations in the case of 21 species of fish. According to Weihs (1973) schooling appears to exert a 'calming' effect and there may be further hydrodynamic energy

Table 2. Changes in assimilation efficiency with growth in different salinities.

Time (days)	4.11‰	10.21‰	16.31‰	22.41‰	28.51‰
15	97.61%	94.66	87.99	62.50	61.38
25	95.17	94.74	89.97	83.77	70.33
35	95.10	96.01	89.02	82.72	73.63
45	95.00	93.88	73.79	89.62	88.68
65	90.36	88.48	86.13	89.03	90.14
75	87.54	89.07	84.13	87.96	92.46
85	85.15	88.02	86.75	91.78	95.00

Table 3. Changes in growth efficiency with time in different salinities.

Period	4.11‰	10.21‰	16.31‰	22.41‰	28.51‰
11-21	6.74	6.44	5.65	5.25	4.94
21-31	2.68	3.79	4.50	2.22	3.69
31-41	2.95	3.41	5.60	3.43	3.39
41-51	8.55	7.09	4.02	8.33	9.44
61-71	6.82	6.72	7.45	8.59	11.99
71-81	9.10	9.53	9.97	9.61	10.17
81-91	12.68	12.94	13.75	13.70	14.25

Table 4. Changes in satiation as percentage of wet body weight with growth in different salinities.

Time (days)	4.11‰	10.21‰	16.31‰	22.41‰	28.51‰
15	26.48	31.57	29.05	29.58	38.41
25	32.43	32.29	29.88	30.00	34.88
35	31.90	34.80	35.54	37.98	46.52
45	31.17	34.95	35.57	34.64	45.18
65	33.36	27.61	36.05	43.98	56.55
75	35.16	26.00	48.48	52.55	57.39
85	34.42	26.53	48.85	53.16	58.05

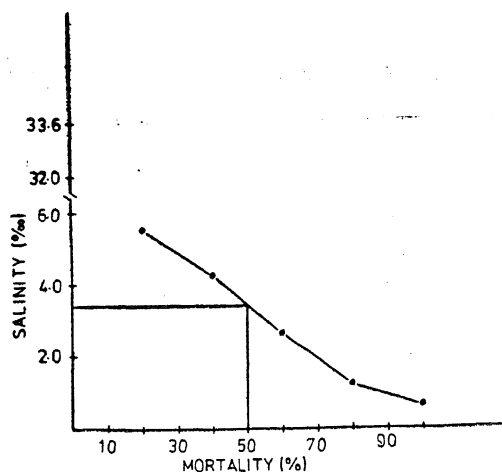


Figure 5. 48 hr mortality rates of juveniles in different salinities.

economics. However, the 'size hierarchy' of Brown (1957) or 'growth depensation' of Ricker (1958), i.e., dominant fish tend to monopolise food and show better growth, even though apparent, was not taken into account in the present study since the fish showing retarded growth were discarded from the growth studies.

The satiation amount as percentage of wet body weight ($3.2-84.3$ mg) ranged from 26.48% - 58.05% for the 91-day experiment period at $26 \pm 2^\circ\text{C}$. Davis and Warren (1968) found young chinook salmon *Onchorhynchus tshawytscha* (0.6 g) would consume 20% body weight/day and Krivobok (1953) as cited by Winberg (1956) obtained daily rations as high as 54% dry weight in very young carp (0.016 g). Brett (1971) computed a daily intake of 30% dry weight at 15°C for one gram sockeye fry.

By the time the fry reach a length of about $17-18$ mm they move towards the main body of the lake (Nair 1957). In the present study, by the time the fry reach this length (61 days), the fish show much better growth in the two higher salinities and better shoaling habits too and hence may start moving up the lake towards the bar-mouth seeking the optimum salinity gradients in the niche. The capture of early juveniles ($22-27$ mm) and their salinity tolerance level ($5.5-33.6\text{‰}$) strengthen the above conclusion.

Using the regression formula for growth in length in the 28.51‰ ($Y = 0.1577 X + 8.4895$), the fish may reach a length of about 6.6 cm during the 0-1 year period and about 4.8 cm in 7-8 months time. Thus the 0-year class individuals of the species mainly contribute to the annual fishery in the estuaries as also noted by Nair (1957) and Raman et al (1975) and start breeding towards the end of the 0-1 year period. It is also quite possible that with their increased preferences to higher salinities with growth, the adult fish may escape into the sea when the bar-mouth is open contributing to the marine stock. Nair (1957) and Raman et al (1975) have noted the seaward migration of this species and Raman et al report that the adult fish migrate to the sea and grow to larger sizes there.

Thus in *C. gymnocephalus* an euryhaline species, the fry show preferred salinity gradients for optimum growth within the fluctuating salinity regime at a stable temperature of $26 \pm 2^\circ \text{C}$ in the laboratory, while similar salinity bound emigrations have been noted in the natural waters by other workers.

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References

- Brett J R 1971 Satiation time, appetite and maximum food intake of sockeye salmon (*Onchorhynchus nerka*); *J. Fish. Res. Bd. Canada* **28** 409-415
- Brown M E 1957 Experimental studies on growth; In *The Physiology of Fishes* (ed.) M E Brown (New York: Academic Press) **1** 361-400
- Canagaratnam P 1959 Growth of fishes in different salinities; *J. Fish. Res. Bd. Canada* **16** 121-130
- Canagaratnam P 1966 Growth of *Tilapia mossambica* Peters in different salinities; *Bull. Fish. Res. Sta. Ceylon* **9** 47-50
- Davis G E and Warren C E 1968 Estimation of food consumption rates; in *IBP Handbook No. 3 Methods for assessment of fish production in freshwaters* (ed.) W E Ricker (Oxford: Blackwell Scientific Publication) pp. 204-225
- Garrison R L 1965 Coho salmon smolts in ninety days; *Prog. Fish-Cult.* **27** 219-230
- Gibson M B and Hirst B 1955 The effect of salinity and temperature on the preadult growth of guppies; *Copeia* **3** 241-243
- Gunther 1961 Salinity and size in marine fishes; *Copeia* **2** 234-235
- Holliday F G T 1971 Salinity-Fishes; In *Marine ecology* (ed.) O Kinne (New York: John Wiley) Vol. **1** Part II pp. 997-1083
- Kapoor B G, Smith H and Verighina I A 1975 The alimentary canal and digestion in Teleosts in *Advances in Marine Biology* (eds.) F S Russel and Sir M Yonge (London: Academic Press) **13** 109-239
- Kinne O 1960 Growth, food intake and food conversion in a euryplastic fish exposed to 2 different temperatures and salinities; *Physiol. Zool.* **33** 288-317
- Nair G S 1957 On the breeding habits and development of *Ambassis gymnocephalus* (Lac.); *Bull. Cent. Res. Inst. Univ. Travancore* **5** 69-76
- Otto R G 1971 Effects of salinity on the survival and growth of presmolt coho salmon (*Onchorhynchus kisutch*); *J. Fish. Res. Bd. Canada* **28** 343-349
- Otto R G and McInerney J E 1970 The development of salinity preference in presmolt coho salmon (*Onchorhynchus kisutch*); *J. Fish. Res. Bd. Canada* **27** 793-800
- Paloheimo J E and Dickie C M 1966a Food and growth of fishes. II Effect of food and temperature on the relation between metabolism and body weight; *J. Fish Res. Bd. Canada* **23** 869-908
- Paloheimo J E and Dickie L M 1966b III. Relation among food, body size and growth efficiency; *J. Fish. Res. Bd. Canada* **23** 1209-1248
- Parker F R 1973 Reduced metabolic rates in fishes as a result of induced schooling; *Trans. Am. Fish Soc.* **102** 125-131
- Raman K, Kaliyamurthy M and Rao G R M 1975 Studies on the biology of *Ambassis gymnocephalus* (Lac.) from Pulicat and Vembanad lakes; *Matsya* **1** 49-52

- Ricker W E 1958 Handbook of computations for biological statistics of fish populations ; *Fish. Res. Bd. Canada Bull.* **119** 1-300
- Warren C E and Davis G E 1967 Laboratory studies on the feeding bioenergetics and growth of fish ; In *The biological basis of freshwater fish production* (ed.) S D Gerking (London : Blackwell Scientific Publications) pp. 175-214
- Weatherly A H 1972 *Growth and ecology of fish populations* (London and New York : Academic Press) p. 293
- Webb P W 1978 Partitioning of energy into metabolism and growth ; in *Ecology of freshwater fish production* (ed.) S D Gerking (London : Blackwell Scientific Publications) pp. 184-214
- Weihs D 1973 Hydrodynamics of fish schooling ; *Nature (London)* **241** 290-291
- Winberg G G 1956 Rate of metabolism and food requirements of fishes ; Belorussian State University Minsk *Fish Res. Bd. Canada Transl. Ser. No.* **194** (1960) p. 251

A comparative study on the mineral composition of the poultry cestode *Raillietina tetragona* Molin, 1858 and certain tissues of its host

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Abstract. The amounts of cations Ca, P, Na, K, Cu and Zn in *Raillietina tetragona* (Cestoda) and in liver, intestinal tissues and blood serum of its host (*Gallus gallus domesticus*) were determined using spectrophotometry, titrimetry, flame photometry and atomic absorption spectrophotometry. Quantitative variations were observed in the distribution of these minerals in the immature, mature and gravid regions of the worm, on dry weight basis. There was a gradual decrease in Ca content of worm along the antero-posterior axis. The Na content, on the other hand showed a reverse trend with the greatest amount in the gravid proglottids. The immature region contained the highest levels of P, K and Cu. The worms showed significantly higher levels of Ca, P, Cu and Zn than the liver and intestinal tissues on dry weight basis. *R. tetragona*, like host liver and intestinal tissues (but unlike blood serum), had quantitative excess of K over Na and other cations.

Keywords. Mineral composition ; poultry cestode ; *Raillietina tetragona* ; host tissues.

1. Introduction

Most of the earlier studies on the biochemistry of cestodes have dealt extensively with their organic constituents, especially the carbohydrates, lipids and proteins. More recently several attempts have been made to identify and quantify the inorganic contents of tapeworms (Salisbury and Anderson 1939 ; Wardle and McLeod 1952 ; Goodchild *et al* 1962 ; Nadakal *et al* 1975 ; Singh *et al* 1978 ; Jakutowicz and Korpaczewska 1979). The data available so far are largely concerned with the larval cestodes and so little is known about the inorganic composition of the adult cestodes. Hence a study was designed to throw some light on the mineral composition of a cosmopolitan poultry cestode, *Raillietina tetragona* and certain tissues of its host, by way of comparison.

2. Materials and methods

Day-old white leghorn chicks were procured and maintained in the laboratory on a basal diet adequate in all nutrients. When three weeks old, 20 healthy birds

of uniform weight were selected. 25 cysticercoids of *Raillietina tetragona* recovered from naturally infected ant vectors (Nadakal *et al* 1971) were administered *per os* to each of the 20 birds. Three weeks post-infection, blood was collected from the wing veins for obtaining serum and then the birds were autopsied. The intestines were split and the worms carefully recovered. The liver, intestines and the worms were washed thoroughly in distilled water and blotted dry with low ash filter paper. 100 worms were pooled and each worm was cut into immature, mature and gravid regions. 40 worms were set apart and sampled as whole worms. The tissue samples were immediately processed for biochemical estimations of ionic Na, K, Ca, P, Cu and Zn. For estimations of Ca and P, 5 samples of each tissue were taken. Each sample was divided into 2 weighed portions. One part was extracted with 10% trichloro-acetic acid for Ca and P determinations and the other part was used for determining percentage of dry matter. For Na, K, Cu and Zn estimations 5 samples from the pooled tissues were dried at 80–100° C. Measured quantities of these dried tissues and serum were ashed separately and extracted with concentrated nitric acid and diluted with glass distilled water, the diluted extracts being used for the estimation of Na, K, Cu and Zn.

Ionic Ca and P were determined following the methods of Clark and Collip (1925) and Fiske and Subba Row (1925) respectively. Na and K were estimated using a flame photometer (Elico Pvt. Ltd., CL 22A), while Cu and Zn were determined using an atomic absorption spectrophotometry (Unicam, SP 1900).

The data obtained for the different regions of the worm were statistically analysed using student's *t* test for the probability of significance of difference between means. The data for the whole worms were compared with those for the host liver and in-testinal tissues and blood serum. *P* values at 5% level are considered to represent significant differences.

3. Results

Quantitative findings for percentages of Ca, P, Na, K, Cu and Zn in the three different regions of the worm are shown in table 1 and those in the whole worms and in the host tissues and blood serum are presented in table 2.

There was a gradual decrease in the Ca content of the worm along the antero-posterior axis. The Ca content of whole worms was 2.64 times and 7.9 times greater than those in liver and intestine, respectively. The immature region contained the greatest amount of phosphorus. The phosphorus content in whole worms was 1.27 times and 2.53 times greater than those in liver and intestine, respectively. A gradation in the amount of Na was observed along the antero-posterior axis of the worm; the peak value being noticed in the gravid region. The whole worms contained 2.53 times less Na than that in the liver. The contents of K and Cu were highest in the immature region. The K content in the worms was twice as much as that in the intestinal tissues and less than half as much as that in liver. The amount of Cu in the worms was considerably less than the amount of Zn. The worms contained significantly higher levels of Cu and Zn than the liver and intestinal tissues. The worms had a quantitative excess of K over the other cations studied.

Table 1. Percentages of Ca, P, Na, K, Cu and Zn in dry weight of *Raillietina tetragona*.

		Region		
		Immature	Mature	Gravid
Ca	mean \pm SE	0.099 \pm .006	0.082 \pm .003	0.066 \pm .005
	P values	<0.05*	<0.05**	<0.002***
P	mean \pm SE	0.184 \pm .013	0.110 \pm .009	0.122 \pm .013
	P values	<0.002*	>0.1**	<0.01***
Na	mean \pm SE	0.091 \pm .009	0.120 \pm .008	0.156 \pm .012
	P values	<0.05*	<0.05**	<0.01***
K	mean \pm SE	0.538 \pm .018	0.472 \pm .021	0.498 \pm .016
	P values	<0.05*	>0.1**	<0.1***
Cu	mean \pm SE	0.008 \pm .0008	0.005 \pm .0005	0.004 \pm .0005
	P values	<0.01*	>0.1**	<0.002***
Zn	mean \pm SE	0.036 \pm .004	0.021 \pm .001	0.035 \pm .003
	P values	<0.01*	<0.01**	>0.1***

Probability of significance of difference between : * immature and mature ; ** mature and gravid ; *** gravid and immature.

4. Discussion

The importance of inorganic substances to adult cestodes is often demonstrated by experimental studies involving mineral deficiencies in the host's diet (Chand 1969 ; Deo and Srivastava 1962 ; von Brand 1966 ; Mathur and Pande 1969 ; Nadakal *et al* 1975). Ca deficiency in the diet of the host birds, for instance, leads to dwarfing of the tapeworm *Raillietina cesticillus* (Mathur and Pande 1969) and dwarfing and reduction in the ash and Ca contents of *R. tetragona* (Nadakal *et al* 1975). These findings indicate that the amount of mineral components of these worms depends on the nutritional condition of the host. In the present study, since the host birds were maintained on a basal diet containing sufficient amount of all the essential nutrients, the mineral levels shown by the worms may be considered to be normal.

A sizeable quantity of mineral components of cestodes is known to be incorporated in the calcareous corpuscles (Scott *et al* 1962 ; von Brand 1966). Large numbers of calcareous corpuscles have been reported in *R. tetragona* (Chowdhury and Singh 1978). The variations observed in the quantitative distribution of the minerals along the antero-posterior axis of *R. tetragona* may reflect a metabolic gradient that might exist in the strobila.

The pattern of distribution of calcium in the three different regions of *R. tetragona* is in conformity with that in *Hymenolepis diminuta* as reported by Goodchild *et al* (1962). The decrease in Ca content in the gravid proglottids may be correlated with the loss of muscular contraction in this region. Shedding of gravid proglottids may be facilitated by reduction in Ca content posteriorly, since its absence or scarcity affects the integrity of intercellular cement substances (Heilbrunn 1952)

Table 2. Percentages of Ca, P, Na, K, Cu and Zn in dry weights of whole worms (*Raillietina tetragona*) and certain tissues of its host (*Gallus gallus domesticus*).

		Tissues		
		Whole worms	Liver	Intestine
Ca	mean \pm SE	0.087 \pm .004	0.033 \pm .004	0.011 \pm .002
	P values	<0.001*		<0.001**
P	mean \pm SE	0.139 \pm .005	0.110 \pm .012	0.056 \pm .008
	P values	<0.05*		<0.001**
Na	mean \pm SE	0.112 \pm .01	0.284 \pm .012	0.098 \pm .011
	P values	<0.001*		>0.1**
K	mean \pm SE	0.501 \pm .008	1.198 \pm .192	0.261 \pm .029
	P values	<0.01*		<0.001**
Cu	mean \pm SE	0.006 \pm .0003	0.004 \pm .0004	0.003 \pm .002
	P values	<0.01*		<0.001**
Zn	mean \pm SE	0.031 \pm .002	0.018 \pm .002	0.017 \pm .001
	P values	<0.001*		<0.001**
		Blood serum		
Ca	mg/100 ml	11.588 \pm .636		
P	mg/100 ml	4.866 \pm .237		
Na	mg/ml	3.210 \pm .124		
K	mg/ml	0.190 \pm .012		
Cu	μ g/ml	0.200 \pm .014		
Zn	μ g/ml	0.706 \pm .028		

Probability of significance of difference between : * worm and liver ; ** worm and intestine.

The higher phosphorus content in the immature region may be attributed to higher metabolic activity in this region. Singh *et al* (1978) observed a significantly higher level of phosphorus in the mature region of *Thysanotia giardi* than in its gravid region. The Ca:P ratios in the worms were higher than those in the liver and intestine.

A gradual decrease in Na content along the antero-posterior axis observed in *R. tetragona* has also been noticed in *H. diminuta* (Goodchild *et al* 1962). The reasons for this regional difference in distribution is not known. The immature region contained the greatest amount of K. Potassium, being the major 'base' of the body cells, may subserve the general functions relating to osmotic pressure regulation and acid-base balance. The tissues of *R. tetragona* like liver and intestinal tissues but unlike the serum, showed quantitative excess of K over Na and other cations. Goodchild *et al* (1962) reported a similar situation in *H. diminuta*. The K:Ca ratios in the worms were considerably lower than those in liver and intestinal tissues, but higher than those in blood serum.

Copper and Zinc are co-factors associated with a number of enzymes including oxidative enzymes, several dehydrogenases, phosphatases and cytochrome oxidases.

Appreciable amounts of these enzymes in the cestode body have been demonstrated (Smyth 1969 ; Enigk *et al* 1976 ; Vasilev *et al* 1976). The higher concentration of Cu and Zn in the immature region of *R. tetragona* may possibly be due to the higher enzymatic activity in this region.

Enigk *et al* (1976) found considerably higher levels of electrolytes in the cyst fluid than in the blood plasma of host and Greichus and Greichus (1980) observed statistically different concentration of minerals in *Ascaris lumbricoides* and the tissues of its host. The presence of higher amounts of cations in *R. tetragona* than in the tissues of its host birds may be due to an efficient selective absorption mechanism prevailing in this worm. Apparently an equilibrium between the parasites and the host tissues with respect to the minerals was not discernible.

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References

- von Brand T 1966 *Biochemistry of parasites* (New York : Academic Press) 1-429
- Chand K 1939 The effects of certain drugs and mineral deficiencies on helminths of ruminants; *Indian J. Vet. Sci.* **9** 267-278
- Chowdhury N and Singh A I 1978 Role of calcareous corpuscles in the organisation of egg pouches in *Raillietina* spp.; *Z. Parasitenkd.* **56** 309-312
- Clark E P and Collip J B 1925 A study of the Tisdall method for the determination of blood serum calcium with a suggested modification; *J. Biol. Chem.* **63** 461-464
- Deo P G and Srivastava H D 1962 Studies on the effects of different deficient diets on the natural resistance of chickens to *Ascaridia galli* (Schrunk) Freeborn; *Indian J. Vet. Sci.* **32** 54-69
- Enigk K, Feder H and Dey Hazra A 1976 Mineral content and enzyme activity of *Cysticercus tenuicollis* in the sheep and pig; *Zbl. Vet. Med.* **B23** 255-264
- Fiske C H and Subba Row Y 1925 The colorimetric determination of phosphorus; *J. Biol. Chem.* **66** 375-400
- Goodchild C G, Dennis E S and Moore J D 1962 Flame photometric studies of helminths : Calcium, Magnesium and Sodium in *Hymenolepis diminuta*; *Exp. Parasitol.* **12** 107-113
- Greichus A and Greichus Y A 1980 Identification and quantification of some elements in the hog roundworm *Ascaris lumbricoides* suum and certain tissues of its host; *Int. J. Parasitol.* **10** 89-92
- Heilbrunn L V 1952 *An outline of general physiology* (Philadelphia: W B Saunders).
- Jakutowicz K and Korpaczewska W 1979 Determination of copper concentration in 7 parasite species by atomic absorption spectrophotometry; *Bull. Acad. Pol. Sci. Ser. Sci. Biol.* **27** 67-70.
- Mathur S C and Pande B P 1969 *Raillietina cesticillus* and *R. tetragona* infections in chicks reared on normal and deficient feeds—an experimental study; *Indian J. Anim. Sci.* **39** 115-134

- Nadakal A M, Mohandas A, John K O and Muralcedharan K 1971 Resistance potential of certain breeds of domestic fowl exposed to *Raillietina tetragona* infection. 3. Species of ants as intermediate hosts of certain fowl cestodes ; *Poult. Sci.* **50** 115-118
- Nadakal A M, Mohandas A, John K O and Simon M 1975 Resistance potential of certain breeds of domestic fowl exposed to *Raillietina tetragona* infections. XII. Effects of calcium deficient diet of the host on *Raillietina tetragona* infections ; *Rev. Parasitol.* **36** 41-46
- Salisbury L F and Anderson R J 1939 Concerning the chemical composition of *Cysticercus fasciolaris* ; *J. Biol. Chem.* **129** 505-517
- Scott D B, Nylen M V, von Brand T and Pugh M H 1962 Mineralogical composition of the cestode calcareous corpuscles of *Taenia taeniaeformis* ; *Exp. Parasitol.* **12** 445-458
- Singh B B, Singh K S, Ghosal A K and Dwarakanath P K 1978 Inorganic calcium, magnesium and phosphorus in *Thysaniezia giardi* ; *Indian J. Parasitol.* **2** 37-38
- Smyth J D 1969 *The physiology of cestodes* (Edinburgh : Oliver and Boyd) 43-51
- Vasilev I, Krusteva O and Gorchilova L 1976 Enzymo-histochemical studies of the tegument of certain species of *Raillietina* genus ; *Khel'mintologiya* **2** 42-49
- Wardle R A and McLeod J A 1952 *The zoology of tapeworms* (Minneapolis : University of Minnesota Press) 99-104

A comparison of the electrophoretic haemoglobin pattern of the commensal rodent species

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Abstract. The present paper reports the haemoglobin pattern by paper electrophoresis of seven rodent and one insectivore commensal species collected from Bombay-Pune region. Almost all the samples possess 1/1 type of haemoglobin which is slower in mobility than that of the normal human type. While the genus *Bandicota* possesses polymorphic haemoglobin types, it is quite surprising that *Suncus murinus* has the haemoglobin of anodic mobility as against its *Soricidae* counterpart's, *Sorex's*, haemoglobin showing cathodic mobility.

Keywords. Electrophoresis ; haemoglobin pattern ; commensal species.

1. Introduction

Use of haemoglobin due to its species specificity has been introduced in taxonomy by modern workers. Of interest to the taxonomists, is the frequent occurrence of genetically controlled multiple haemoglobins in wild species ; these may be population, species or genus characteristics. While dealing with 324 mammalian and 300 vertebrate species, Johnson (1974) and De Smet (1978) showed the similarities and the differences in the mobility of the haemoglobin patterns of various species. The polymorphism could be located even at the lowest level of the taxonomic groups. In India haemoglobin studies have revealed many variants in man and in domestic animals (Sukumaran 1975; Naik *et al* 1969 ; Naik 1975). Wild rodent populations have yet not been touched so far by the Indian taxonomists to study the comparative account of the haemoglobin patterns by the electrophoretic techniques. The present article is an initial attempt to report the haemoglobin types of some of the Indian commensal rodent species.

2. Materials and methods

Sixtytwo specimens belonging to seven rodent and one insectivore species were collected from the various residential localities and godown areas of Bombay and Pune cities. Live rats were caught by a number of methods, like trapping, cynogassing, etc., with the help of workers of the Municipal Corporations. The animals were sacrificed by cutting their heads on the spot of collection and the blood samples

were collected in the heparinised tubes. The identification of rats was done at ZSI, WRS, Pune.

Haemoglobin solution was prepared and subjected to paper electrophoresis following the method of Naik *et al* (1969) and Wright (1974) with some modifications. The buffer used for the studies was Barbitone (pH 8.6) supplied by M/s Centron Research Laboratories, Bombay. The electrophoresis was run for four hours and the strips were studied directly after drying. The anodic mobility of the haemoglobins of different species was recorded and confirmed by repeated runs.

Normal human blood samples (twentyfive in total) were provided, as and when required, by ESIS Hospital Aundh Camp, Pune, for comparison.

3. Results and discussion

The diagrammatic representation shown in figures 1 and 2 of haemoglobins of commensal rodent species clearly indicates the occurrence of Hb-1/1 type of haemoglobin in these rats except in *Bandicota bengalensis kok* which shows subspecific polymorphism. The nomenclature for haemoglobins is given according to Johnson (1974). No minor or trailing fractions could be located in these species. Hb-1/1 type of haemoglobin has already been reported in the Euresian rodent species except in those of the genera like *Peromyscus* and *Apodemus* (Johnson 1974). It also appears from the present studies that the haemoglobins of most of the species belonging to the genera, *Rattus*, *Mus* and *Bandicota*, show relatively slower mobility than that of the normal human haemoglobin (HbA). However, Johnson (1974) has reported the equal mobility for normal human and European *R. rattus* haemoglobins. *R. r. wroughtoni* possesses faster moving haemoglobin than that of *R. r. rufescens* and has the same mobility as that of the normal human type. That means there is a difference even at the protein level in these two sympatric subspecies. Tiwari *et al* (1971) who awarded the specific status to *rufescens* get the support from the different Hb patterns of these rats. However, the haemoglobins in the species like *R. norvegicus* and *Mus musculus* show the same mobility as that of *R. r. rufescens*. Thus, further studies have become necessary for the taxonomic confirmation of various species and subspecies of the genus *Rattus*.

Haemoglobin of the insectivore, *Suncus murinus*, quite surprisingly showed the same mobility as that of the normal human type (figure 1). It is interesting to note that while Johnson (1974) has reported cathodic migration of haemoglobins for most of the insectivore species, including those of the genus *Sorex*, the present observations show the anodic mobility for *Suncus murinus*. Confirmation and further studies on the haemoglobins of the order *Insectivora* will also be interesting.

No common type of haemoglobin could be traced for any of the bandicoot species under the present studies. All the species possess multiple haemoglobins. Taking the present findings as sample drawn at random the probabilities of multiple haemoglobins for the seven rodent and one insectivore species can be roughly estimated to 50 %. De Smet (1978), while comparing the haemoglobins of approximately 300 vertebrate species, reported 40% occurrence of multiple haemoglobins in the order *Rodentia*. He has also pointed out that the existence of intra-subspecific haemoglobin polymorphism is a common phenomenon. If the slow moving

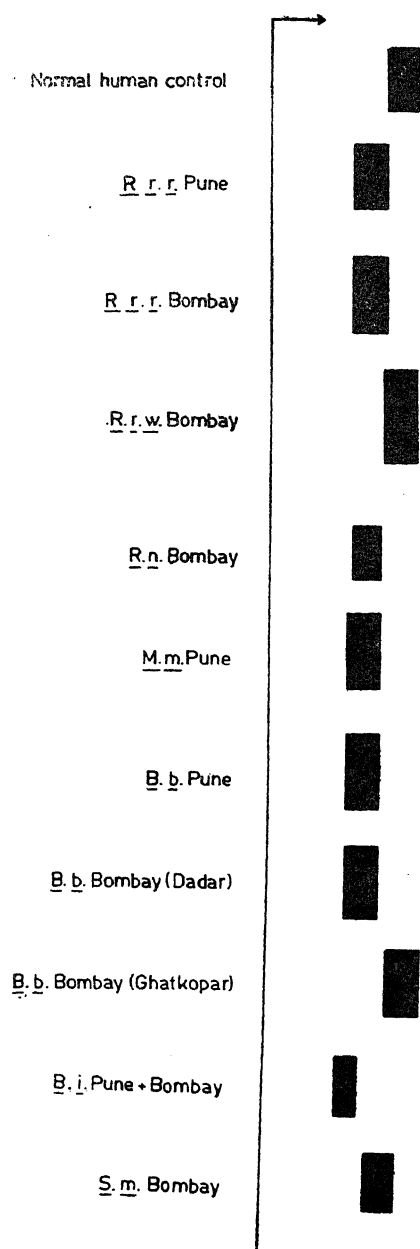


Figure 1. Diagrammatic representation of the haemoglobin pattern of seven rodent and one insectivore species collected from Bombay-Pune region.

haemoglobin band of *B. b. kok* is included, it will be seen that this type of haemoglobin is a common type found in all the three genera under the present study. If all the rodent species are studied, an evolutionary trend of rodent haemoglobin could be unravelled.

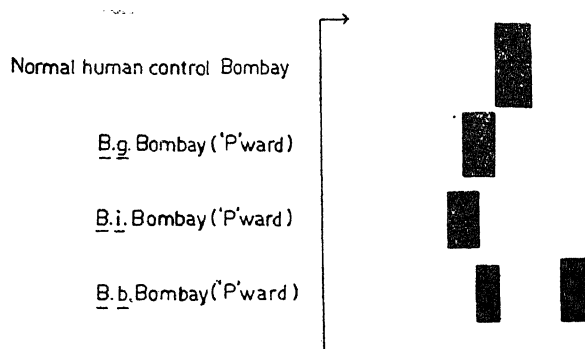


Figure 2. Diagrammatic representation of the genus *Bandicota* haemoglobins showing the heterozygous form trapped from Malad, Bombay.

The *B. b. kok* populations caught from Bombay-Pune region possess the multiple haemoglobins with a mixing of the two genotypes (figure 2). The populations from Bombay city ward (Dadar) and Pune city possess slow moving haemoglobin, while the other collected from a distant suburb (Ghatkopar) on NE side of the Bombay city has the fastest moving haemoglobin. The animal with heterozygous haemoglobin depicted in figure 2 was caught from another distant suburb (Malad) on NW side of the Bombay city. Existence of the different homozygous alleles for the haemoglobins in the separate populations and also of the heterozygous form in the subspecies indicates their genetic control over the two polymorphic haemoglobins. So, if the allelic variation at the genetic loci controlling the structure of haemoglobin in the *kok* populations is studied further in detail, it might be possible to estimate the degree of heterozygosity in the populations. This evidence can be supported by estimating the degree of variations in the other proteins also. Selander *et al* (1969) have reported a wide range of genetic variations in the degree of differences in the wild populations of European house mouse. As all the proteins are genetically controlled, the effect of these degree of differences on the morphotaxonomy of the above-mentioned subspecies will be studied in detail in future.

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References

- De Smet and Willem H C 1978 A comparison of the electrophoretic haemoglobin pattern of the vertebrates; *Acta Zool. Pathol. Antverp.* **70** 119-131
- Johnson M L 1974 Mammals. In : *Biochemical and immunological taxonomy of animals* (ed.) C A Wright (London : Academic Press) pp. 1-87
- Naik S N 1975 Haemoglobin polymorphism in Indian domesticated and wild ruminants; *Indian J. Hered.* **7** 23-30
- Naik S N, Sukumaran P K and Sanghvi L D 1969 Haemoglobin Polymorphism in Indian Zebu cattle; *Heredity* **24** 239-247
- Selander R K, Grainger Hunt W and Suh Y Yang 1969 Protein polymorphism and genetic heterozygosity in two European subspecies of the house mouse; *Evolution* **23** 379-390
- Sukumaran P K 1975 Abnormal haemoglobins in India. In : *Trends in haematology* (eds.) N N Sen and A K Basu, J B Chatterjee Memorial Committee, Calcutta, School of Tropical Medicine, Calcutta, India
- Tiwari K K, Ghosh R K and Chakraborty S 1971 Notes on a collection of small mammals from Western Ghats, with remarks on the status of *Rattus rufescens* (Gray) and *Bandicota indica malabarica* (Shaw); *J. Bomb. Nat. Hist. Soc.* **68** 378-384
- Wright C A 1974 (ed.) *Biochemical and immunological taxonomy of animals*, 1st Edition (London : Academic Press) pp. 490 + xii.

Abbreviations

<i>R. r. r.</i>	..	<i>Rattus rattus rufescens</i>
<i>R. r. w.</i>	..	<i>Rattus rattus wroughtoni</i>
<i>R. n.</i>	..	<i>Rattus norvegicus</i>
<i>M. m.</i>	..	<i>Mus musculus</i>
<i>B. b.</i>	..	<i>Bandicota bengalensis</i>
<i>B. i.</i>	..	<i>Bandicota indica</i>
<i>B. g.</i>	..	<i>Bandicota gigantea</i>
<i>S. m.</i>	..	<i>Suncus murinus</i>



Studies on egg and nymphal parasites of rice planthoppers, *Nilaparvata lugens* (Stål) and *Sogatella furcifera* (Horvath)*

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Abstract. Three species of egg parasites, viz., *Anagrus* sp., *A. optabilis* (Mymaridae) and *Oligosita* sp. (Trichogrammatidae), and a nymphal/adult parasite *Gonatopus* sp. of rice planthoppers were studied for their biology and control potential. Larger number of adult mymarids emerged from host eggs between 8.30 a.m. and 12.30 p.m. of the day whereas trichogrammatid adults emerged between 12.30 p.m. to 4.30 p.m. All the three species parasitised both brown planthopper (BPH) and white backed planthopper (WBPH) but, in general, failed to parasitise rice leafhoppers.

Developmental duration from oviposition to adult emergence noted for these parasites indicated that males of mymarids, in general, developed faster (10-11 days) than females (12-13 days) at 20-32° C prevailing during October, whereas *Oligosita* females developed more slowly (14-15 days). However, both *A. optabilis* and *Oligosita* developed three days faster at 30-38° C prevailing during April. Fecundity in terms of number of eggs parasitised per female varied from 12.3 to 20.3. Under greenhouse conditions, release of 1 and 5 pairs of mymarid parasites for 10 days reduced the nymphal hatch of BPH by 60 and 85%, respectively. Nymphal/adult parasite *Gonatopus* sp. completed its life cycle in 19.5 to 31 days on both BPH and WBPH. While the 4th and 5th instar nymphs of BPH were parasitised more frequently, green leafhopper nymphs were not parasitised. Besides being endoparasitic, the adult females also predated on and killed as many as 5.2 nymphs a day.

Keywords. *Anagrus* spp. *Gonatopus* ; parasitoids ; rice planthoppers.

1. Introduction

Rice planthoppers have gained major pest status causing 'hopper burn' in several rice growing Asian countries. Outbreaks of brown planthopper *Nilaparvata lugens* (Stål) have been reported in different parts of India (Kalode 1974 ; Kulshrestha *et al* 1974). Also, white backed planthopper, *Sogatella furcifera* Horvath, is noted to cause damage in northern India (Verma *et al* 1979), while smaller brown planthopper, *Laodelphax striatellus* (Fallen) has been reported from the Punjab (Shukla 1979).

Twentyfour species of egg parasites and 30 species of nymphal parasites have been recorded mainly on brown planthopper (BPH), besides three species of nematode parasites, 11 species of pathogenic fungi and 61 species of insect and spider predators are also reported (Anonymous 1978 ; Manjunath 1978 ; Manjunath *et al* 1978 ; Chiu 1979).

From India, except for a brief account of *Anagrus* sp. (Samal and Misra 1978), no detailed study seems to have been made of egg or nymphal/adult parasites of rice planthoppers. In the present investigation 3 species of egg parasites, viz., *Anagrus* sp., *Anagrus*? *optabilis* (Perkins) (Mymaridae) and *Oligosita* sp. (Trichogrammatidae) and nymphal adult parasite, *Gonatopus* sp. (Dryinidae) were investigated with respect to their biology, host range and biocontrol potential against the planthoppers. *A. optabilis* has been reported here for the first time from India, while genus *Gonatopus* on BPH is a new record.

2. Materials and methods

2.1. Rearing

2.1a *Egg parasites*: Rearing was initiated with the parasitised eggs collected from rice plants in the field and glasshouse. Leaf sheaths of plants were peeled out and kept in glass jars containing a little water at the bottom and covered by an inverted glass funnel. A glass tube was kept inverted on the glass funnel. Adults emerging from host eggs through leaf sheaths moved upwards and were collected in the glass tubes. Populations of parasites were further built up by exposing new plants to parasites on which eggs had been freshly laid and later keeping such plants in jars for adult collection (figure 1). All the three species of parasites were reared together. Only for experimental purpose adult parasites were differentiated under binocular microscope. Except when specified, rearing and biology studies were carried out in the laboratory at room temperature ranging from 20–38° C and, humidity varying from 30 to 80% RH.

2.1b *Nymphal parasite* : Parasite pupae from affected brown planthopper culture in glasshouse were collected in tubes to initiate rearing. Emerging *Gonatopus* adults were fed with honey solution, and mated females were released on T(N)1 rice plants along with the nymphs of BPH and WBPH. Adult parasites were transferred every day to fresh plants with healthy nymphs kept in separate cages to avoid nymphal mortality due to predation. All the studies were conducted in the glasshouse with host insects reared on T(N)1 plants at $30 \pm 5^\circ \text{C}$ and $80 \pm 10\% \text{RH}$.

2.2. Experiments

Studies on egg parasites were carried out using individual rice seedlings (15–20 day old) kept in test tubes. Gravid females of BPH/WBPH were confined in such tubes for 24 hr for oviposition prior to the release of parasites. After 6–7 days plants were dissected to count number of eggs parasitised and such eggs were then kept on moist filter paper in glass tubes with screw caps to observe adult emergence.



Figure 1. Laboratory rearing method for the egg parasites of rice planthoppers *Nilaparvata lugens* and *Sogatella furcifera*. Adult planthoppers are released on T(N)1 plants for oviposition (pot 1), followed by parasite release for parasitisation (pot 2). After healthy planthopper eggs hatch (pot 3), plants are cut to be kept in adult emergence jars to collect the emerging adults.

Life cycle of nymphal parasite, *Gonatopus*, was studied by offering planthopper nymphs to mated females confined on rice plants in mylar film cages. Similar set-up was used for experiments to determine suitable nymphal instar for parasitization, host range and to estimate predation by adults.

3. Results

3.1. Egg parasites

3.1a *Adults*: Mymarid adults (*Anagrus* spp.) are brown in colour with fringed wings, body measuring 0.7 to 0.9 mm in length, while *Oligosita* is greenish yellow in colour with smaller body of about 0.5 mm with rounded wings. However, *Anagrus* sp. has bulkier abdomen with short ovipositor whereas *A. optabilis* has a slender abdomen with long and prominent ovipositor. In the laboratory population of mymarids, females were more numerous than males (sex ratio of 5 females : 1 male). The virgin females parasitised host eggs normally thus indicating thelytokous development. Adults of mymarids lived for 24–36 hr without any food and those of *Oligosita* survived for 24–48 hr. Males of *Oligosita* were not observed and hence this species appears to be uniparental.

Adult emergence patterns were noted for the three species by recording the number of adults emerging during different time intervals of the day. Results, illustrated in figure 2, indicated more than 70% of mymarid adults emerged between 8.30 a.m. and 12.30 p.m., whereas maximum percentage of trichogrammatid adults emerged between 12.30 p.m. and 4.30 p.m.

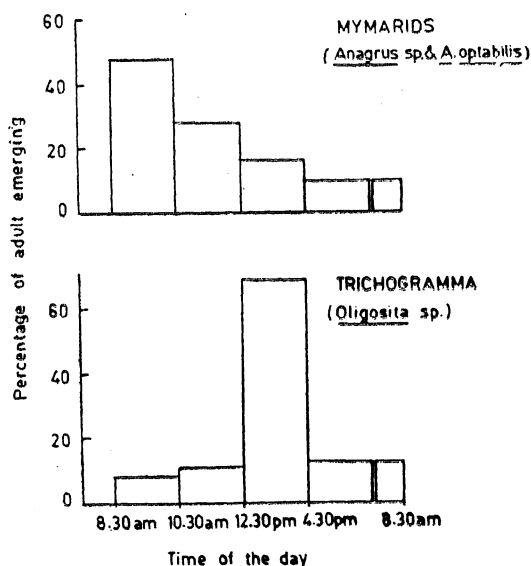


Figure 2. Adult emergence patterns for mymarid (*Anagrus* sp. and *A. optabilis*) and trichogrammatid (*Oligosita* sp.) egg parasites of rice planthoppers *Nilaparvata lugens* and *Sogatella furcifera*. It may be noted that while mymarids emerge before noon, trichogrammatids emerge during afternoon.

3.1b *Developmental duration and fecundity*: The entire life cycle of egg parasites was completed inside the host egg. All three species parasitised the eggs of both brown planthopper and white backed planthopper. Eggs parasitised by mymarids turned yellow by day 7, assuming orange to red-brown colour by day 8 and 9 when pupation occurred inside the eggs. Those parasitised by *Oligosita* turned greenish yellow by 7-8th day without further change in colour.

The data on duration of development, presented in table 1, indicated that males of mymarids, in general, developed faster (10-11 days) than females (12-13 days) while the *Oligosita* females developed slowly (14-15 days) at 20-32° C prevailing during October 1979. However, both *A. optabilis* and *Oligosita* developed 3 days faster at 30-38° C prevailing during April 1980. A female mymarid could parasitise 15-20 planthopper eggs whereas that of the *Oligosita* parasitised 12-18 eggs.

3.1c *Host range* : As noted above the 3 species of parasites readily parasitised both the species of rice planthoppers. However, they failed to parasitise eggs of rice leafhoppers, viz., *Nephotettix* spp., *Inazuma dorsalis* and *Tettigella spectra*.

3.1d *Biocontrol potential* : An experiment was conducted under glasshouse conditions to note the potential of mymarid parasites in checking egg hatching of brown planthopper. One pair of newly emerged brown planthopper was first caged on T(N)1 plants followed by daily release of one and five pairs of *Anagrus* parasites into the cage from day 5 to day 15. As indicated in table 2, release of 1 pair of mymarids reduced the egg hatch by about 60%, while release of 5 pairs reduced it by 85% indicating a high biocontrol potential of the egg parasites.

Table 1. Developmental duration and fecundity of egg parasites of planthopper *Nilaparvata lugens* (BPH) and *Sogatella furcifera* (WBPH).

Parasite	Host	Development duration* (days)		Fecundity (no. of eggs parasitised/ female)
		Male	Female	
<i>Anagrus</i> sp.	BPH	13.5	12.6	20.3
	WBPH	...	10.8	14.5
<i>A. optabilis</i>	BPH	11.0	12.1 9.2**	17.5
	WBPH	10.4	12.1	19.0
<i>Oligosita</i> sp.	BPH	...	13.8 10.6**	18.0
	WBPH	...	15.3	12.3

* Development observed during October 1979 (temp. ranging 20.1 to 31.8° C) and

** April 1980 (29.1 to 38.0° C).

Table 2. Influence of mymarid parasites (*Anagrus* spp.) on hatching of *Nilaparvata lugens* eggs.

Treatment	No. of eggs hatched/ female* Mean \pm SE	% reduction in viability
Control	439.3 \pm 50.3 ^a	...
1 pair of parasites released from day 5 to 15	199.3 \pm 25.7 ^b	59.5
5 pairs of parasites released from day 5 to 15	66.2 \pm 11.4 ^c	84.9

* mean of 6 replications.

comparison of means : a-b ; a-c and b-c, $p < 0.001$ (t -test).

3.2. Nymphal parasite

3.2a Adults : Adult female *Gonatopus* resembles an ant in appearance but can be distinguished from the latter by the presence of chelate fore tarsi adapted for catching the prey. While the females are apterous (figure 3), males have membranous wings and are more active than females. Body size is smaller (2 to 3 mm in length) in the case of males than in females which measure 4 to 5 mm in length, and have a dark black body. Adult longevity ranged from 7 to 10 days for males and 15 to 20 days for females when provided with honey solution.

Life cycle of *Gonatopus* was studied by offering nymphs of both **BPH** and **WBPH** to mated females for oviposition and later observing these nymphs periodically. The female parasite holds 4th or 5th instar planthopper nymphs with its forelegs, bends its abdomen and thrusts eggs into the host body (figure 3). The parasitised nymphs are immobilised for 2-3 min before they move off. Since eggs are laid internally, incubation period could not be exactly determined, but small black or yellow sac-like structures (larval sacs) appear on the abdomen of nymphs 3-5 days after oviposition (figure 3). The larval sacs, one or two per nymph, containing larvae enlarge gradually. At the end of the larval period, ranging from 7 to 12 days, the larval sac bursts and a small 2-4 mm long yellowish white or sometimes pinkish larva crawls out killing the host. Prepupal stage lasts for 12 to 24 hr and pupation takes place either on the rice plant or on the sides of cages. Prior to pupation, the larva secretes an yellowish-white fluid to form a membranous, oval shaped puparium (figure 3). Adults emerge after 9 to 12 days. The total life cycle of *Gonatopus* takes 19.5 to 31 days on **BPH** and 24.5 to 31 days on **WBPH** (table 3).

Since the dryinid normally selects only older nymphs or adult hosts for oviposition, suitability of different nymphal instars for parasitisation was studied. As presented in table 4, maximum parasitisation (larval sacs) per female was observed

Table 3. Life cycle of nymphal parasite, *Gonatopus* sp. on *Nilaparvata lugens* and *Sogatella furcifera*.

Host	Incubation period (days)	Larval period (days)	Pre-pupal period (hours)	Pupal period (days)	Total developmental duration (days)
<i>N. lugens</i>	3-5	7-12	12-24	9-13	19.5-31.0
<i>S. furcifera</i>	3-5	10-12	12-24	11-13	24.5-31.0

Table 4. Parasitisation of different instar nymphs and adults of *Nilaparvata lugens* by *Gonatopus* sp.

Host instar	No. of insects studied*	No. of parasites released	No. of nymphs parasitised	Av. No. of nymphs parasitised/female/day
1st instar nymph	80	8	0	0
2nd instar nymph	80	8	0	0
3rd instar nymph	80	8	8	1
4th instar nymph	80	8	40	5
5th instar nymph	80	8	48	6
Adults	80	8	24	3

* based on four replications at 20 insects/replication.

in 5th instar nymphs (av. number 6) followed by 4th instar nymphs (5) and adults (3).

3.2b Host range : Host range and preference for parasitisation by *Gonatopus* was noted by offering 4th and 5th instar nymphs of BPH, WBPH and green leafhoppers (*Nephotettix* spp.). It is evident from table 5; that *Gonatopus* preferred BPH to WBPH since 13 out of 30 BPH nymphs were parasitised as against 9 of WBPH, while it did not parasitise green leafhopper nymphs.

3.2c Nymph predation : Besides parasitisation of older nymphs, *Gonatopus* females were also observed to predate on younger nymphs. The observation on the extent of nymphal predation showed that one female could kill on an average 5.2 nymphs per day. These findings revealed that dryinid, *Gonatopus*, had a good biocontrol potential against planthoppers both as nymphal/adult parasite as well as nymphal predator.

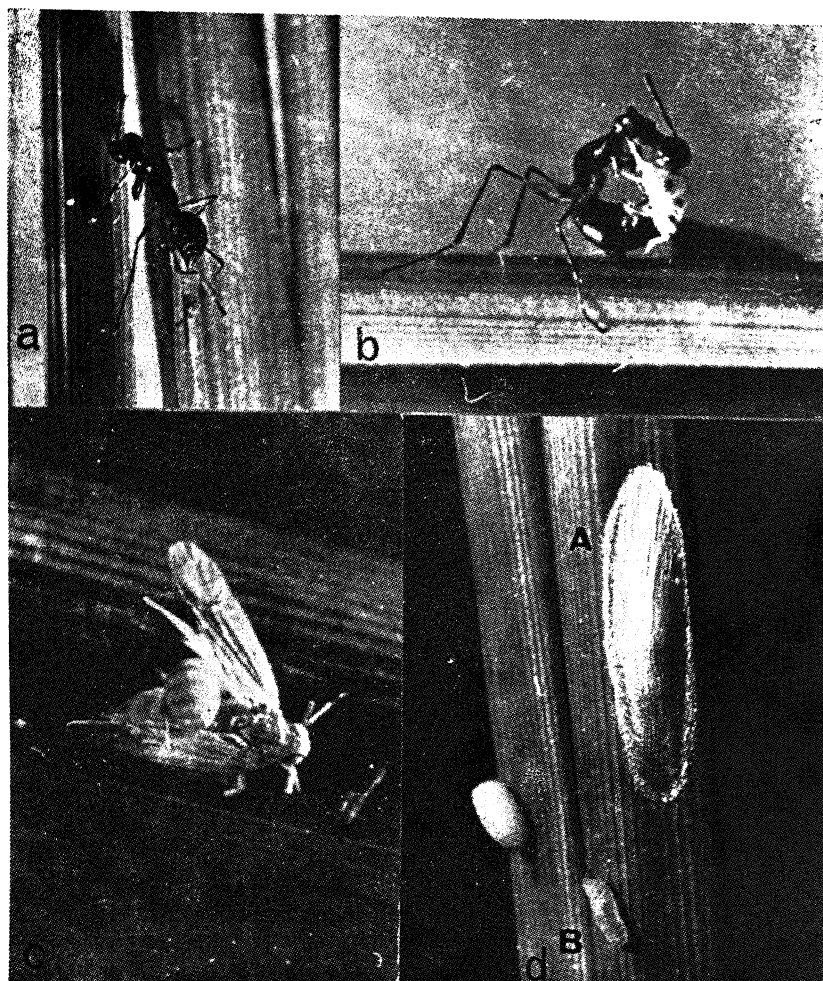


Figure 3. Nymphal/adult parasite (*Gonatopus* sp.) of rice planthoppers, *Nilaparvata lugens* and *Sogatella furcifera*. a. Apterous adult female; b. a prey being held by a female for oviposition; c. an adult planthopper with larval sac; d. fully grown larva (B) and pupa in puparium (A).

Table 5. Host range and preference for parasitisation by *Gonatopus* sp.

Host	No. of host insects studied*	No. of parasites released	Cumulative number of insects parasitised after...	
			3 days	5 days
Brown planthopper	30	15	10	13
White backed plant- hopper	30	15	5	9
Green leafhopper	30	15	0	0

* Total of 3 replications at 10 insects/replication.

4. Discussion

Adult emergence pattern has been noted for *Paracentrobia andoi*, a trichogrammatid egg parasite of the leafhopper, *Nephotettix cincticeps* (Vungsilabutr 1978). Most of the adults emerged between 8 a.m. and 12 noon. In the present work, while mymarids had a peak emergence before noon, trichogrammatid adults emerged during afternoon. Samal and Misra (1978) noted the development period for *Anagrus* sp. to be 12 to 14 days during April–May with temperatures ranging from 24.4 to 35° C. Our results for *Anagrus* sp. show that the developmental period was 11 to 14 days during October. The parasite took lesser time to develop on WBPH than it did on BPH. It is not known, however, if these two species of parasites are the same or different. Temperature effects on the rate of development have been elaborately investigated for *P. andoi* (Vungsilabutr 1978). Though in the present study constant temperatures were not maintained, differences in developmental duration observed for *A. optabilis* and *Oligosita* sp. noted during October (temp. ranged from 20.1 to 31.8° C) and April (29.1 to 38° C) essentially reflect the effect of temperature.

Though all the three species readily parasitised both BPH and WBPH eggs, mymarids failed to parasitise leafhoppers. The preference of mymarid parasites among the planthoppers and host range of the trichogrammatid are yet to be studied in detail. It is not uncommon, however, for an egg parasite to have both leaf and planthopper hosts as noted for *P. andoi* (Vungsilabutr 1978) and many other species (Anonymous 1978).

Studies on *Gonatopus* sp. substantiate the view that dryinids would make good agents for biological control of injurious *Cicadellidae* and *Fulgoridae* (Olmi 1976).

Biological control of rice planthoppers through the use of natural enemies has so far not been attempted in field scale. However, the only suggested candidate for this purpose—the egg-nymphal predator *Cyrtorhinus lividipennis* has a wider range of predation (Bentur and Kalode 1980). In contrast, the egg parasites and

the nymphal parasite investigated in the present work have host range restricted only to brown planthopper and white backed planthopper and also possess a good degree of control potential. They can also be considered for use in field along with *C. lividipennis*. Nevertheless, development of economical mass rearing methods, information on the behaviour of released population under field conditions and knowledge of mutual interaction of natural enemies are prerequisites for any such attempts.

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References

- Anonymous 1978 Prospects for biological control of rice hoppers—A status paper, Commonwealth Institute of Biological Control, p. 12
- Bentur J S and Kalode M B 1980 Biocontrol studies on leaf and planthoppers. Proc. 3rd Workshop of All India Coordinated Research Project on Biological Control of Crop Pests and Weeds, Ludhiana, Indian Council of Agricultural Research, New Delhi, pp. 103–108
- Chiu S G 1979 Biological and cultural control of the brown planthopper pp. 335–355. In: *Brown planthopper : Threat to rice production in Asia*, International Rice Research Institute, Los Banos, Philippines
- Kalode M B 1974 Recent changes in relative pest status of rice insects as influenced by cultural, ecological and genetic factors; *Intern. Rice Res. Conference*, April 1974, IRRI, Los Banos
- Kulshrestha J P, Anjaneyulu A and Padmanabhan SY 1974 The disastrous brown planthopper attack in Kerala; *Indian Farm* 24 5–7
- Manjunath T M 1978 Two nematode parasites of rice brown planthopper in India; *Int. Rice Res. Newslett.* 3 11–12
- Manjunath T M, Rai P S and Gowda G 1978 Parasites and predators of *Nilaparvata lugens* in India; *PANS* 24 265–269
- Olmi M 1976 Experience and prospects of biological control with Dryinidae (Hymenoptera : Bethyloidea) In: *Atti XI Congresso Nazionale Italiano di Entomologia* (English Abstract) pp. 371–373
- Samal P and Misra B C 1978 Notes on egg parasites of the brown planthopper *Nilaparvata lugens* (Stål) in Orissa; *Oryza* 15 96–98
- Shukla K K 1979 Occurrence of a new insect, small brown planthopper, *Loadelphax striatellus* (Fallen) in India; *Curr. Sci.* 48 548
- Verma S K, Pathak P K, Singh B N and Lal M N 1979 Occurrence of brown and whitebacked planthoppers in Uttar Pradesh, India; *Int. Rice Res. Newslett.* 4 20
- Vungsilabutr P 1978 Biological and morphological studies of *Paracentrobia andoi* (Ishii) (Hymenoptera : Trichogrammatidae), a parasite of the green leafhopper, *Nephotettix cincticeps* Uhler (Homoptera : Deltocephalidae); *Esakia* 11 29–51

New natural enemy complex of some fulgoroids (Insecta: Homoptera) with biological studies of three hymenopterous parasites (Insecta : Hymenoptera)

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Abstract. Natural enemy complex of the planthoppers, *Dichoptera hyalinata* F., *Eurybrachys tomentosa* F., and *Ricania fenestrata* F. includes two nymphal ectoparasites (*Dryinus* spp.), two egg parasites (*Proleurocerus fulgoridis* F. and *Tetrastichus* sp.), an internal larval mermithid parasite, and a predator (*Phidippus* sp.). Biological aspects of *Dryinus* spp. and *P. fulgoridis* are discussed.

Keywords. Fulgoroidea ; natural enemies ; parasite ; predator.

1. Introduction

The planthoppers, an economically very important group as pests and vectors of plant diseases, were investigated in relation to their association with crops as well as their natural enemies. In India the bionomics and effectiveness of the natural enemies of fulgoroids were studied extensively with reference to *Pyrilla* spp. (Rahman and Ramnath 1940 ; Rahman 1941 ; Sen 1948 ; Narayanan and Kundanlal 1953 ; Subba Rao 1957), *Nilaparvata lugens* Stål (Abraham *et al* 1973 ; Kalode 1976 ; Manjunath 1978 ; Manjunath *et al* 1978a,b ; Rai and Chandrasekhar 1979 ; Samal and Misra 1975, 1978a,b) and *Sogatella furcifera* Horvath (Chaudhury and Ramzan 1968 ; Israel and Prakasa Rao 1969). The present paper brings to light the occurrence of new natural enemies of the fulgoroids, *Eurybrachys tomentosa* F. (Eurybrachidae, Fulgoroidea), *Ricania fenestrata* F. (Ricanidae, Fulgoroidea) and *Dichoptera hyalinata* F. (Dictyopharidae, Fulgoroidea), the first two being pests of important crops such as *Santalum album*, *Zizypus jujuba*, *Cajanus indicus*, *Calotropis gigantea*, *Camellia sinensis*, *Gossypium* spp., *Jasminum flexile*, etc. (Chatterjee 1933 ; Hutson 1919 ; Light 1929 ; Puttarudriah and Maheswariah 1958). Besides, the biology of two ectoparasites, *Dryinus* spp. (Dryinidae, Bethyloidea) and an egg parasite, *Proleurocerus fulgoridis* F. (Encyrtidae, Chalcidoidea) are discussed.

Material and methods

Parasitized eggs (in the case of egg parasites) and nymphs (in the case of ectoparasites) were brought from the field and reared in the laboratory for adult emergence. The emerged adult parasites were caged in small glass chimneys (110 ml) or vials (10 ml, 15 ml) (figure 1A), and were fed with a dilute sucrose solution on cotton, the latter being fixed on a wire projecting inside the containers. Laid egg masses of *E. tomentosa* and fresh nymphs of *E. tomentosa* and *R. fenestrata* were provided in the cages for the egg parasites and ectoparasites respectively to enable the parasites to lay their eggs. From the day of parasitization one egg from the same batch of parasitized eggs was dissected out daily to observe the sequence of changes in the life cycle of the egg parasites. Parasitized nymphs were caged separately and the larval development of the ectoparasites observed. The larvae of the ectoparasites emerging from the hosts were reared to pupate on a glass surface (figure 2C), which enabled observation of their development. Laboratory conditions during the present study were 19° C-temperature and 60%-90% (relative humidity).

Observations

Incidence of new parasites and predators

Nymphs of *D. hyalinata* were parasitized by an ectoparasite, *Dryinus* sp. (A)* (figure 1A), while another dryinid, *Dryinus* sp. (B)*, was recorded on the nymphs of *E. tomentosa*. The eggs of *E. tomentosa* were parasitized by an encyrtid, *Pteromalus fulgoridis*, and an eulophid, *Tetrastichus* sp., while the adults of this species were parasitized by a larval mermithid. In the case of *R. fenestrata* a predator, *Phidippus* sp., was recorded as a natural enemy (figures 1B to 1F). Observations on the seasonal cycles of the parasites (figure 3) revealed that *Dryinus* sp. (A) occurred in the field for 7 months, *P. fulgoridis* for 5 months, *Dryinus* sp. (B) for 3 months, and *Tetrastichus* sp. for a month. *Dryinus* sp. (A) reached the peak of its activity during November followed by the absence of the nymphal population during the four succeeding months, and their parasitism was minimum during July. *P. fulgoridis* was very active during February-March, when all the egg masses of *E. tomentosa* collected from the field were found to be parasitized by this parasite. Parasitization by *Tetrastichus* sp. was also occurring only during April. Both *P. fulgoridis* and *Tetrastichus* sp. parasitized the same egg mass of *E. tomentosa* and in one instance the former parasitized one egg of an egg mass, the latter 39 eggs. Occurring on its host only during February-March, the ability of *Dryinus* sp. (B) to suppress the population of its host, *E. tomentosa* appeared to be less pronounced than that of *Dryinus* sp. (A) on its host *D. hyalinata*. Adults of *E. tomentosa* affected by a larval mermithid parasite were also identified and the parasitized adults appeared pale and

* *Dryinus* spp. have been designated as (A) and (B) as they have been identified to two new species (Dr Z Boucek, Commonwealth Institute of Entomology, London—personal communication). Being very host specific the identity of the species (A) and (B) is not confusing.

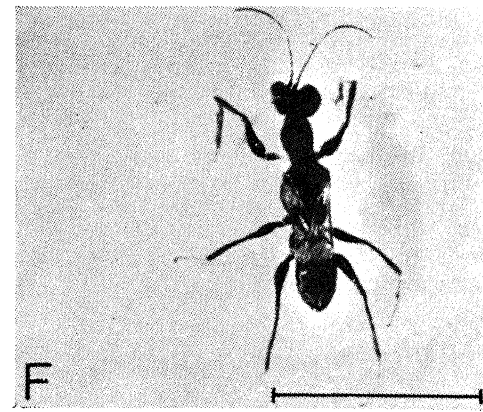
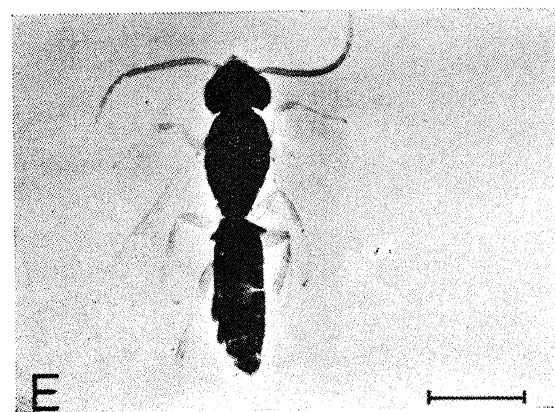
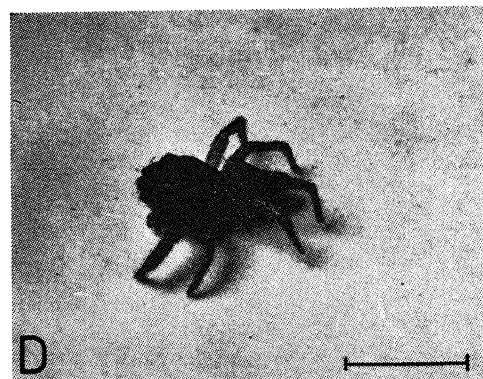
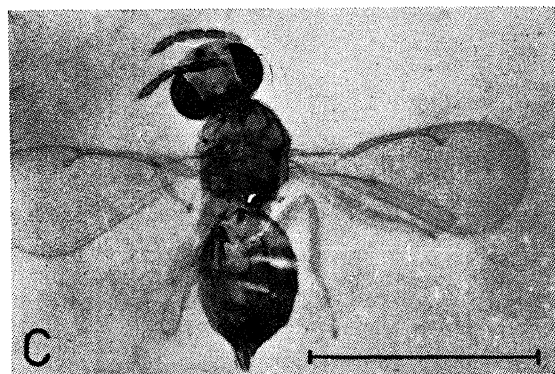
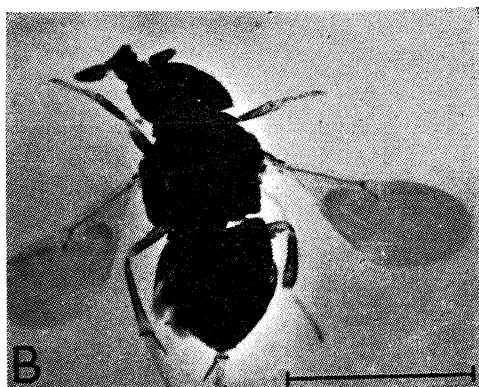
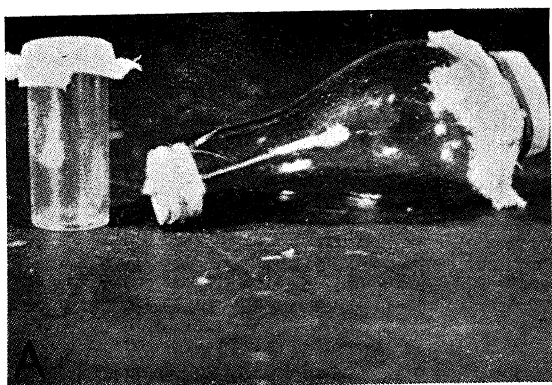


Figure 1. A. Rearing cages for the parasites, B. *Proleurocerus fulgoridis*, C. *Tetrastichus* sp., D. *Phidippus* sp., E. *Dryinus* sp.(B). Male, F. *Dryinus* sp. (A)—Female (B, C, E—Scale = 1 mm ; D, F—Scale = 5 mm).

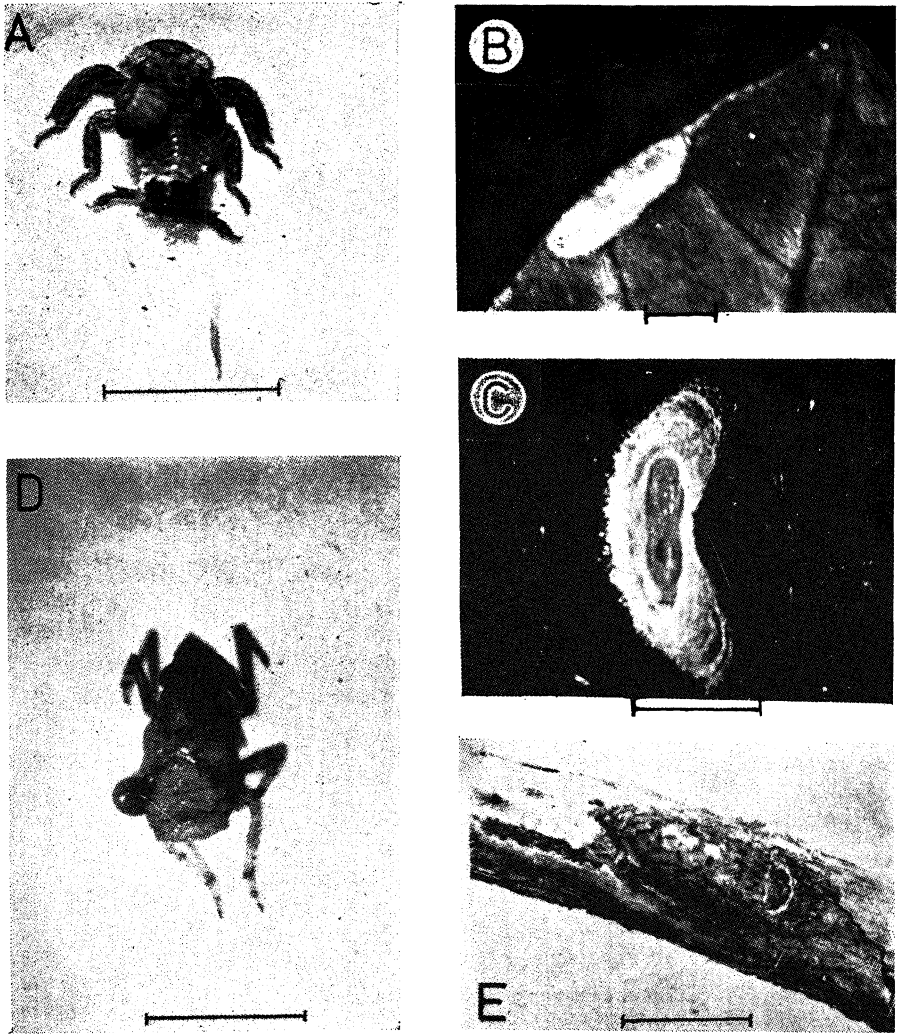


Figure 2. A. Parasitized *E. tomentosa* nymph showing thalacium. B, C. Pupa of *Dryinus* sp. (B). D. Parasitized *D. hyalinata* nymph showing thalacium. E. Pupa of *Dryinus* sp. (A) (Scale = 5 mm).

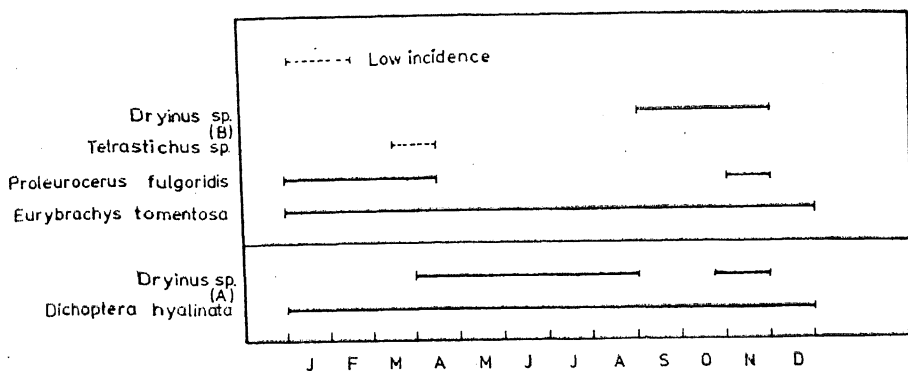


Figure 3. Seasonal cycle of host and parasites.

seemed distinctly inactive. All the life stages of *R. fenestrata* except eggs were actively predated upon by the spider, *Phidippus* sp. (Salticidae, Arachnida) in the field as well as under laboratory conditions. Besides feeding on *R. fenestrata*, *Phidippus* sp. also fed on other insects found in the same habitat, but less frequently.

3.2. Biology of parasites

3.2a *Dryinus* sp. (B) :—The adult parasites with a shining black body were observed in the field actively moving around the plants in search of their host. When the parasite actively chased the host for oviposition, a distinct parasitic behaviour was noticed (Swaminathan and Ananthakrishnan 1981). Mostly second and third instars of the host were preferred for parasitization by the females, while in the laboratory they were able to parasitize the first, second, and third instars, and sometimes the fourth instar nymphs as well. The fifth instar nymphs of the hosts easily escaped from the attack of the parasite by kicking and jumping. In all the first instar host nymphs examined, the egg-deposition and development of the 'thelacium' were noticed only under the hind wing pad, while in the second and third instar host nymphs, parasitization was under both the wing pads. In the fourth instar nymphs, the parasite larva failed to develop after a certain stage; even in cases when it successfully completed its delayed larval life, and left the host, it finally died before spinning the cocoon. The average time taken to complete oviposition increased as the size of the host increased in the successive instars, being 95 sec, 117.9 sec, 172.5 sec, and 235 sec in first, second, third, and fourth instars respectively. The time interval between two successive acts of oviposition was 5–10 min. Eggs were usually laid beneath the wing pads and in the laboratory each host carried only one egg. *Dryinus* sp. (B) female laid more than one egg/host under different wing pads if the same host was exposed for a long time and no other hosts were available. A minimum of 1 egg/host and a maximum of 2 eggs/host were recorded in the laboratory, but in the field the host nymphs generally showed only one egg.

The eggs were elongate, cylindrical and translucent. In dryinids the first larval instar is spent entirely within the host (Clausen 1940). Three to four days after

oviposition the parasite developed a bag-like structure (figure 2A) at the oviposited site on the host's body. This bag-like structure the 'thalachium', was suggested to be formed by a proliferation of the host integument (Subba Rao 1957). The cyst membrane of the thalachium is found all over the parasite larva thus preventing direct contact of the larva with the body cavity of the host and the larva derives all food material through this membrane (Clausen 1940). The whitish and translucent thalachium gradually turned brown after 3 days. The parasite larva developed inside the thalachium slowly sucking the haemolymph of the host without affecting the latter's life activities. With the establishment of the thalachium on host, the planthopper nymphs lost their ability to moult further, particularly when the nymphs were parasitized at the end of their stadial period. In the laboratory only in three instances two larvae were found to develop on a single host. Such parasites developed one on either side or on the same side under the wing pads. If two thalachia developed from the same host, only one parasite larva fully completed its development and successfully pupated while the other showed partial development ultimately perishing along with the host. Within the thalachium the larva underwent three moults, and the mature larva sucked out most of the haemolymph of the host before leaving it. The resultant enlargement caused a cleavage line antero-posteriorly in the thalachium, through which the emerging parasite larva crawled out and dropped down to the substratum. Owing to extensive feeding by the escaping larva, the host suffered excessive shrinkage and died. The mature larva (2.5-4 mm long and 0.75-1 mm wide) appeared dull white in colour with a pointed anterior end and bulging posterior end. It soon started building a white cocoon on the leaves or stems (figure 2B). In the laboratory, the cocoon spinning was also noticed on the wall of the glass containers (figure 2C) and cloth, within 5 min of escape from the host. While spinning the pupal case with white silken threads secreted from the mouth, the larva entered the cocoon by peristaltic movements. The large well-developed mandibles were efficiently used in cocoon building, particularly in cutting the threads. The fully formed cocoon was generally oval, double-walled—a tightly-spun inner and loosely-spun outer wall—measuring on an average 7 mm long and $2\frac{1}{2}$ mm wide.

Though the parasite larva entered the cocoon immediately after leaving the host, the actual pupation took place only after 4-5 days. During this period the brownish larva showed peristaltic movements inside the cocoon, subsequently turning reddish brown, and resulting in a complete pupa exhibiting swift and frequent back and forth movements for 10 days. On the 7th-8th day after spinning cocoon, wings and limb buds developed and the demarcation of the head, thorax, and abdomen was evident. During the late pupal period there was a deposition of black excretory material in the caudal end of the cocoon. On the 15th day after spinning the cocoon, the pupa turned fully black and there was a cessation of movements.

The active male and female adults emerged from the cocoon by making a hole at the anterior end. Both parthenogenetic and sexual reproduction were observed, the former always resulting in male offspring. Under laboratory conditions the average life span of adult female and male was 25 days and 16 days respectively, the average oviposition period of females being 20 days and the average total number of eggs laid by a single female was 35. The average total duration of egg, larval,

Table 1. Duration of various stages in the life-cycle of parasites (in days).

Species	Period between egg-laying and thalacium formation	Duration of the larval stage spent in thalacium	Period spent in the cocoon	Total life-cycle
<i>Dryinus</i> sp. (A)	3	4	23	30
	4	6	23	33
	2	4	25	31
	6	6	26	38
	6	5	23	34
Mean	4.2 ± 1.6	5 ± 0.89	24 ± 1.27	33.2 ± 2.79
<i>Dryinus</i> sp. (B)	4	8	25	37
	3	8	22	33
	3	9	23	35
	3	12	23	38
	4	8	23	35
Mean	3.4 ± 0.49	9 ± 1.55	23.2 ± 0.93	35.6 ± 1.74
	Egg	Larva	Pupa	Total
<i>Proleurocerus fulgoridis</i>	2	3	4	9
	2	4	5	11
	2	4	4	10
	2	4	9	15
	2	4	10	16
Mean	2 ± 0	3.8 ± 0.4	6.4 ± 2.58	12.2 ± 2.79

and pupal periods was 35.6 ± 1.74 days and the parasite spent more time in the cocoon (23.2 ± 0.98 days) than in the thalacium (9 ± 1.55 days) (table 1).

3.2b *Dryinus* sp. (A) : The life cycle appeared similar to that of *Dryinus* sp. (B) with only some minor variations. The average total duration of egg, larval, and pupal periods was 33.2 ± 2.79 days (table 1). Eggs were laid beneath the wing pads, in the dorsal middle region of the thoracic segments, and on the dorsal lateral region of the abdominal segments. Midthoracic region was highly preferred for egg laying. Each host carried 1-2 parasite larvae both under laboratory and field conditions.

The larvae developed inside the thalacium (figure 2D) which was gelatinous white during the first day of its formation and turned brown after 2-3 days. As in *Dryinus* sp. (B), when parasitized by two larvae, one developed faster than the other and pupated, while the other died with the host. The larva underwent three moults inside the thalacium. While leaving the host, the larva fed on most of the

host haemolymph and escaped from the thalacium by rupturing it while the host was killed. Immediately after leaving the host, the larva formed an oval cocoon with two walls of silken threads on the bark of the host plant (figure 2E). The colour of the cocoon varied with the surrounding and was brownish (on bark in the field) or grey (on cloth in rearing cages in the laboratory). Both in the laboratory and under field conditions the second, third, and fourth instar host nymphs were susceptible to parasite attack. All the behavioural patterns and methods of oviposition were similar to those of *Dryinus* sp. (B).

3.2c *Proleurocerus fulgoridis* : The eggs of this encyrtid parasite were fusiform and stalked. The average length and width of the eggs were 507.5μ and 160.2μ respectively, while the stalk measured 267.0μ . The eggs were laid singly within the host egg. The durations of egg and larval stages were 2 days and 3.8 ± 0.4 days respectively (table 1). On the second and third day of larval development the larva grows to a maximum size by consuming all the contents of the host egg. The fully grown larva measured 1.78 mm long and 0.68 mm wide with 12 segments (figure 4). During the development of the larva of the parasite the host egg showed no colour change. Inside the host egg a constant peristaltic movement of the parasite larva was noticed. Pupation resulted on the 5th or 7th day of parasitization and the pupal period lasted 6.4 ± 2.58 days (table 1). With the pupation of the parasite the colour of the host egg changed to brown. The host nymphs from unparasitized eggs in a partially parasitized egg mass always hatched 1-2 days before the parasites emerged.

The adult parasites (figure 1B) were shiny black and started mating as they emerged from the host egg. The males chased the females and while moving in front of the females, vibrated their half-extended wings. The longevity of the adults was 2-4 days when fed with 5% sucrose solution. The females started laying eggs shortly after emergence. Young host egg masses (2-3 days old) were preferred for oviposition. Usually 1-2 females attacked a single egg mass under field conditions. Each female took 5-6 hr to complete egg laying. As soon as a gravid female located a fresh egg mass of *E. tomentosa*, it moved over it for sometime and then started laying eggs. After making punctures on the waxy coat at many places, the females inserted their ovipositors and laid eggs. Superparasitism was not observed. In the egg masses of *E. tomentosa* 40.59%-100% of the eggs were found parasitized under field condition (table 2).

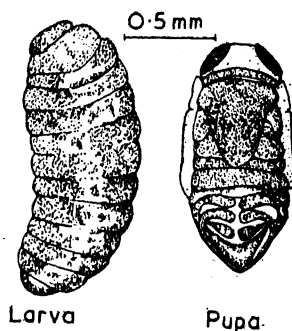


Figure 4. Immature stages of *Proleurocerus fulgoridis*.

Table 2. Percentage parasitization of individual egg masses of *Eurybrachys tomentosa* by *Proleurocerus fulgoridis* under field condition.

Total number of eggs in a mass	Number of parasitized eggs	Number of unparasitized eggs	Percentage of eggs parasitized in a mass
114	111	3	97.36
106	103	3	97.16
101	41	60	40.59
110	110	...	100.00
123	105	18	85.37
100	97	3	97.00
103	102	1	99.02
90	90	...	100.00
91	91	...	100.00
83	83	...	100.00
57	56	1	98.24
98 ± 17.7	89.9 ± 22.4		92.3 ± 17.6

4. Discussion

A high degree of host preference is exhibited by Dryinidae commonly found as parasites of both adults and nymphs of Fulgoroidea and Cicadellidae and they are known to be either solitary or gregarious (Clausen 1940). Observations of Subba Rao on *Lestodryinus pyrrillae* Kieff (1957), Swaminathan and Anantha-krishnan (1981) on two *Dryinus* spp., and the present study shows the following characteristic features of these effective biological control agents: (i) The dryinids are host specific, an important quality for effective biological control agents as suggested by DeBach (1964); (ii) They show preference for nymphal stages; (iii) Oviposition behaviour like chasing, pouncing, and paralysing is exhibited by the females; (iv) The dryinids exhibit arrhenotoky; and (v) The parasitized nymphs are prevented from moulting to the next instar. Similarly other dryinids, *Pseudogonatopus hospes* Perk. (Pagden 1934) and *Dicondylus lindbergi* Heikinheimo (Heikinheimo 1957) were found to prefer the last two instars of *Delphacodes furcifera* Horvath and adults of *Delphacodes pellucida* F. respectively. The present study was confined to the effects of parasitism by the dryinids on nymphs of the host. Hence the effects of parasitism on adult hosts such as deformities in reproductive organs and external sex reversal in males (Clausen 1940) was not studied. There are five larval instars in dryinids (Clausen 1940), of which the first is seen inside the host body, the following three are spent inside the thalacium, the fifth one escaping and crawling away from the host for pupation. In the present investigation also, similar larval instars were noticed including three moults in the thalacium.

No natural enemies have so far been recorded from *E. tomentosa*, *R. fenestrata*, and *D. hyalinata*, the first two being pests of important crops (vide introduction). In *E. tomentosa* all the life stages—eggs, nymphs, and adults are parasitized by the natural enemies. In *R. fenestrata* the eggs are laid inside the plant tissue. Hence the nymphs and adults are alone predated by the spider and in *D. hyalinata* only the nymphal stages are attacked by the natural enemy. All the natural enemies reported here appear to be new records. Under field condition the *E. tomentosa* egg masses were parasitized to a maximum extent (100%) by *P. fulgoridis* which shows the latter to be a promising biological control agent of the former.

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References

- Abraham C C, Mathur K P and Das N M 1973 New record of *Coccinella arcuata* Fabricius (Coleoptera : Coccinellidae) as a predator of *Nilaparvata lugens* Stål in Kerala; *Agric. Res. J. Kerala* 11 75
- Chatterjee N C 1933 Entomological investigations on the spike disease of Sandal (12). The life history and morphology of *Eurybrachys tomentosa* Fabr. Fulgoridae (Hompt.); *Indian Forest Rec.* 18 1-26
- Chaudhary J P and Ramzan M 1968 *Pachygonatopus* sp. (Dryinidae : Hymenoptera). A new parasite of *Sogatella furcifera* Horvath (Delphacidae : Homoptera); *Indian J. Entomol.* 30 317
- Clausen C P 1940 *Entomophagous insects* (New York : McGraw-Hill) pp. 688
- DeBach P 1964 *Biological control of insect pests and weeds* (London : Chapman and Hall) pp. 844
- Heikinheimo O 1957 *Dicondylus lindbergi* sp. n. (Hym. : Dryinidae) a natural enemy of *Delphacodes pellucida* (F.); *Ann. Entomol. Fennici* 23 77-85
- Hutson J C 1919 Some minor insect pests in Ceylon in 1919; *Trop. Agric. Peradeniya* L 3 No 2.
- Israel P and Prakasa Rao P S 1969 Record of *Anagrus* sp., a mymarid (Hymenoptera) parasite on eggs of *Sogatella furcifera* Horvath on rice in India; *Curr. Sci.* 38 320-321
- Kalode M B 1976 Brown planthopper in rice and its control; *Indian Farming* 27 3-5
- Light S S 1929 Report of the Entomologist (for 1928); *Bull. Tea Res. Inst. Ceylon* No. 3
- Manjunath T M 1978 Two nematode parasites of rice brown planthopper in India; *Int. Rice Res. Newslett.* 3 11-12
- Manjunath T M, Rai P S and Gowda G 1978a Parasites and predators of *Nilaparvata lugens* in India; *Pans* 24 265-269, 387
- Manjunath T M, Rai P S and Gowda G 1978b Natural enemies of brown planthopper and green leafhopper in India; *Int. Rice Res. Newslett.* 3 11
- Narayanan E S and Kundanlal 1953 Studies on the chalcid egg parasites of *Pyrilla* sp. occurring in Delhi. Part I. Bionomics of *Tetrastichus pyrrillae* Crawf., *Ageniaspis pyrrillae* Mani, and *Cheiloneurus pyrrillae* Mani; *Indian J. Entomol.* 15 173-179

- Pagden H 1934 Notes on Hymenopterous parasites of padi insects in Malaya ; *Fed. Malay. States. Dept. Agric. Bull.* **15** pp. 13
- Puttarudriah M and Maheswariah B M 1958 *Ricantia fenestrata* Fabricius A potential pest of Cotton ; *Indian Cotton Growing Rev.* **12** 1
- Rahman K A 1941 Parasites of the insect pests of sugarcane in Punjab ; *Indian J. Agric. Sci.* **11** 119-128
- Rahman K A and Ramnath 1940 Bionomics and control of the Indian sugarcane leafhopper, *Pyrilla perpusilla* Wlk. (Rhynchota, Fulg.) in the Punjab ; *Bull. Entomol. Res.* **31** 179-190
- Rai P S and Chandrasekhar H T 1979 The natural enemy complex of rice brown planthopper ; *Curr. Sci.* **48** 23-24
- Samal P and Misra B C 1975 Spiders : The most effective natural enemies of the brown planthopper in rice ; *Rice Entomol. Newslett.* **3** 31
- Samal P and Misra B C 1978a Notes on the egg parasites of the brown planthopper *Nilaparvata lugens* (Stål) in Orissa ; *Oryza* **15** 96-98
- Samal P and Misra B C 1978b *Casnoidea indica* (Thunb) a carabid ground beetle predating on brown planthopper, *Nilaparvata lugens* Stål of rice ; *Curr. Sci.* **47** 688-689
- Sen A C 1948 The *Pyrilla* pest of cane in Bihar and its control by utilization of its natural parasites ; *Indian J. Entomol.* **10** 45-50
- Subba Rao B R 1957 The biology and bionomics of *Lestodryinus pyrrillae* Kieff. (Dryinidae : Hymenoptera) a nymphal parasite of *Pyrilla perpusilla* Walk. and a note on its role in the control of *Pyrilla* ; *J. Bombay Nat. Hist. Soc.* **54** 741-749
- Swaminathan S and Ananthakrishnan T N 1981 Oviposition behaviour in two species of dryinid parasites (Dryinidae, Hymenoptera) ; *Proc. Indian Acad. Sci. (Anim. Sci.)* **90** 113-116

Transabdominal migration of ova in some freshwater turtles

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Abstract. The phenomenon of transabdominal migration of ova is fairly common in all three fresh water turtles, *Lissemys punctata punctata* (70%), *Kachuga tectum tectum* (4%) and *K. smithi* (73%), studied for the present work. Individuals of *Lissemys punctata punctata*, *Kachuga smithi*, showed higher frequency of ovular migration in smaller individuals. It is suggested that a better weight balance is possibly achieved by ovular migration in these aquatic reptiles.

Keywords. *Lissemys punctata punctata* ; *Kachuga tectum tectum* ; *Kachuga smithi* ; transabdominal ; ovaries ; corpus luteum ; ova ; oviduct ; ovulation.

1. Introduction

Upon rupture each ovarian follicle releases its contained egg into the body cavity which is immediately engulfed by the infundibulum of the oviduct. Eggs ovulated by left ovary normally pass into left oviduct and those of right ovary into right oviduct. The collapsed follicular wall of the ovulated follicle eventually gets transformed into corpus luteum. The number of corpora lutea thus provides a fairly accurate index to the number of eggs produced by an ovary of a side at a given time and also indicates the number of eggs expected in the oviduct of that side. Ordinarily, the total number of corpora lutea in the two ovaries of an animal corresponds to the total number of eggs in the two oviducts, except in instances where either oviposition is extended beyond the resorption of corpus luteum or clutching is multiple. Yet, it has often been observed that the counts of corpora lutea in the ovary of one side and the number of oviducal eggs in the ipsilateral oviduct differs, some times strikingly. This difference is sought to be explained only by the phenomenon of transabdominal migration of eggs during the short period that intervenes between the act of ovulation and encapsulation by the oviduct.

Although reported in mammals too (Asdeil 1946; Arey 1954), the phenomenon of transabdominal migration of eggs in reptiles was for the first time reported by Weekes (1935). Ever since, the phenomenon has been reported for snakes (Tinkle 1957), lizards (Tinkle 1961; Mayhew 1963, 1965, 1966, 1971; Telford 1969; Cuellar 1970; Goldberg 1972) and turtles (Tinkle 1957; Legler 1958; Moll and

Legler 1971; White and Murphy 1973; Plummer 1977; Cox and Marion 1978). To obtain some more information on this very common phenomenon among reptiles and therefore presumably of importance to them, three Indian freshwater turtles, *Lissemys punctata punctata*, *Kachuga tectum tectum*, and *K. smithi* were intensively studied from the standpoint of ovulation in them from 1976 to 1978.

2. Materials and methods

Specimens were collected by hand, muddling, or by cast nets from two different sources. *Lissemys punctata punctata* were collected from Lake Mansar (about 65 km in the East of Jammu city, India) and *Kachuga tectum tectum* and *K. smithi* were collected from a slow running stream, New Gho-Manasan Khul, situated about 15 km south-west of Jammu city. The taxonomy of the forms studied was done after Smith (1931).

All linear measurements of the specimens were done in the laboratory with the help of 1 meter flexible steel tape from live animals. Measurements were recorded to the nearest millimeter. After preliminary weighing and measurements, the turtles were dissected for examination of ovarian weight; the number and size of ovarian follicles and corpora lutea; the number, size and weight of shelled oviducal eggs, were noted separately for right and left side. The weights were recorded to the nearest milligram.

3. Results and discussion

During their breeding season, which extends from August to October in *Lissemys punctata punctata*, October to February in *Kachuga tectum tectum* and August to November in *K. smithi*, 14 adults of *Lissemys p. punctata* (table 1, figure 1) 30 of *Kachuga t. tectum* (table 2, figure 2) and 17 individuals of *K. smithi* (table 3, figure 3) were found to contain eggs in their oviducts. Of these 4 individuals of *Lissemys p. punctata*, 25 of *Kachuga t. tectum* and 2 of *K. smithi*, showed more corpora lutea than the number of eggs in their oviducts, representing more than one series of ovulation and were thus of varying size and appearance. The remaining individuals showed number of corpora lutea to be equal to the total number of eggs in two oviducts.

Of the remaining 10 turtles of *Lissemys p. punctata*, three showed the number of eggs in one side oviduct to be equal to the number of corpora lutea in the ovary of the same side. However, in the remaining 7, a striking disparity in their number (table 1, figure 1) was observed. Four of these 7 turtles showed more corpora lutea in the right ovary than in the left and three more corpora lutea in the left ovary than in the right. The number of eggs in the oviducts of these seven was equal on two sides in two individuals (5 and 3 in each oviduct), and unequal in 5, being greater in the right oviduct in 2 and in the left oviduct in 3 individuals.

In *Kachuga t. tectum*, only two individuals of the 5 animals (where number of corpora lutea was equal to egg number in the oviducts) showed the phenomenon of ovular migration (table 2, figure 2). The remaining 3 individuals possessed equal number of corpora lutea and oviducal eggs on each side. In these cases it was impossible to determine whether ova had migrated from one side to the

Table 1. Number of corpora lutea, eggs and ovarian weight (g) in *Lissemys p. punctata* on the right and left sides of the body.

Sl. No.	Plastron length (mm)	Right side			Left side			Total number of corpus luteum	Total number of shelled eggs
		Corpus luteum	Shelled eggs	Wt. of ovary	Corpus luteum	Shelled eggs	Wt. of ovary		
1.	250	3	2	29.400	4	5	48.900	7	7
2.	265	7	4	33.800	11	6	61.100	18	10
3.	225	4	3	10.600	6	2	24.250	10	5
4.	286	5	7	19.600	8	6	30.300	13	13
5.	225	1	2	6.700	5	4	6.450	6	6
6.	245	6	4	56.400	5	7	42.900	11	11
7.	267	5	5	32.000	5	5	36.000	10	10
8.	235	3	3	43.100	5	5	21.050	8	8
9.	281	8	7	21.720	4	5	35.800	12	12
10.	244	6	5	42.000	4	5	31.100	10	10
11.	217	4	3	8.200	2	3	7.720	6	6
12.	282	15	8	18.200	10	5	28.100	25	13
13.	258	9	5	27.200	9	4	20.900	18	9
14.	239	4	4	36.900	4	4	48.200	8	8

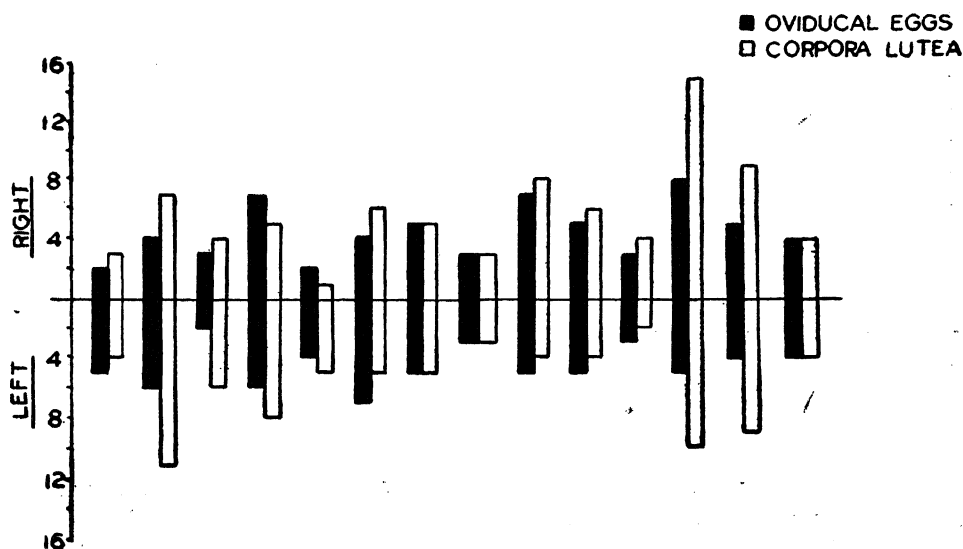


Figure 1. Comparative counts of oviducal eggs and corpora lutea in *Lissemys p. punctata* (represented by black and white bars, respectively).

Table 2. Number of corpora lutea, eggs and ovarian weight (g) in *Kachuga t. tectum* on the right and left side of the body.

Sl. No.	Plastron length (mm)	Right side			Left side			Total number of corpora luteum	Total number of shelled eggs
		Corpus luteum	Shelled eggs	Wt. of ovary	Corpus luteum	Shelled eggs	Wt. of ovary		
1.	170	9	6	12.580	10	4	8.200	19	10
2.	173	3	4	6.600	4	3	19.950	7	7
3.	165	7	3	2.310	5	4	3.425	12	7
4.	166	3	4	3.700	4	3	3.450	7	7
5.	161	5	4	2.900	8	3	2.750	13	7
6.	168	8	4	4.640	14	5	4.150	22	9
7.	160	7	3	2.995	3	3	2.725	10	6
8.	185	3	3	43.200	3	3	26.800	6	6
9.	167	7	2	3.670	4	3	3.810	11	5
10.	166	5	4	4.300	7	3	5.900	12	7
11.	161	7	2	3.995	5	3	6.800	12	5
12.	153	3	3	5.065	3	3	7.050	6	6
13.	173	9	2	5.900	13	2	3.910	22	4
14.	168	4	4	11.700	10	3	20.000	14	7
15.	155	5	4	7.600	8	3	2.820	13	7
16.	161	4	4	3.750	4	4	7.150	8	8
17.	143	2	2	2.190	6	2	2.220	8	4
18.	178	10	6	4.700	11	4	5.000	21	10
19.	152	9	2	3.480	3	3	5.850	12	5
20.	170	5	4	4.300	7	2	3.850	12	6
21.	154	6	3	6.610	5	4	4.115	11	7
22.	157	6	3	2.200	6	3	2.405	12	6
23.	165	9	3	4.425	6	4	5.110	15	7
24.	165	6	3	4.200	7	4	4.280	13	7
25.	183	13	4	7.300	8	4	3.200	21	8
26.	171	7	5	13.000	8	5	4.080	15	10
27.	155	4	3	3.245	5	2	3.225	9	5
28.	160	5	5	4.400	11	5	8.315	16	10
29.	171	7	6	4.425	13	4	9.000	20	10
30.	147	5	2	2.835	5	4	2.715	10	6

other. In the other two cases, more corpora lutea were seen on the left side, when oviducal eggs were more on the side opposite.

Eleven of 15 *K. smithi* showed an extrauterine migration of ova (table 3, figure 3). Of the remaining four, three showed that the count of oviducal eggs

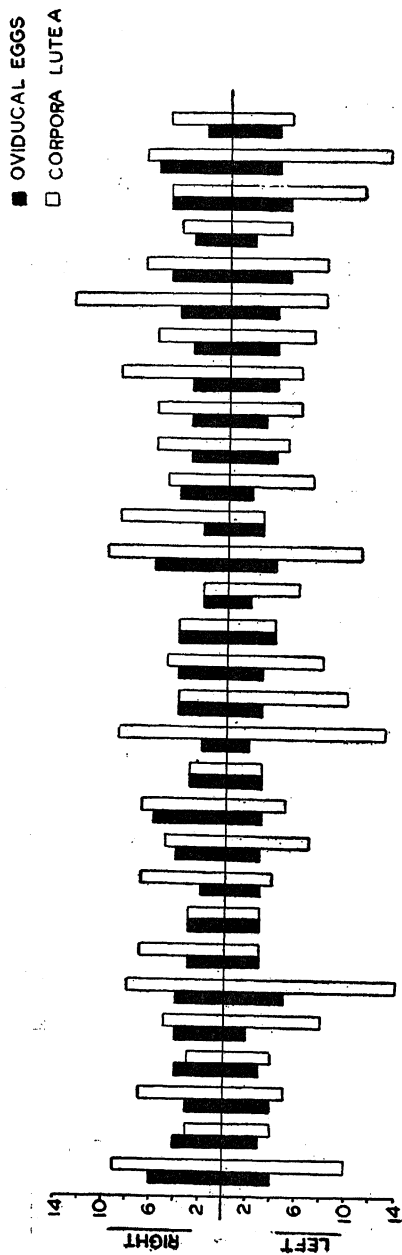


Figure 2. Comparative counts of oviducal eggs and copora lutea in *Kachuga t. tectum* (represented by black and white bars, respectively).

Table 3. Number of corpora lutea, eggs and ovarian weight (g) in *Kachuga smithi* on the right and left side of the body.

Sl. No.	Plastror length (mm)	Right side			Left side			Total number of corpora luteum	Total number of shelled eggs
		Corpus luteum	Shelled eggs	Wt. of ovary	Corpus luteum	Shelled eggs	Wt. of ovary		
1.	173	1	...	26.750	2	3	46.900	3	3
2.	176	7	3	42.750	5	3	18.775	12	6
3.	191	6	3	18.500	...	3	12.950	6	6
4.	160	6	4	17.350	2	4	14.350	8	8
5.	194	4	2	74.350	1	3	38.000	5	5
6.	197	4	4	43.000	3	3	44.100	7	7
7.	190	2	3	23.100	5	4	39.500	7	7
8.	192	4	4	36.450	2	2	41.100	6	6
9.	195	5	3	19.500	1	3	26.100	6	6
10.	199	4	5	29.000	6	5	41.000	10	10
11.	195	4	2	48.210	2	4	30.380	6	6
12.	173	1	3	9.300	3	1	30.200	4	4
13.	180	3	4	43.700	3	2	41.800	6	6
14.	192	3	3	35.510	3	3	22.800	6	6
15.	187	4	5	13.000	7	6	4.600	11	11
16.	193	1	1	14.000	2	2	5.500	3	3
17.	177	6	3	6.580	4	2	4.750	10	5

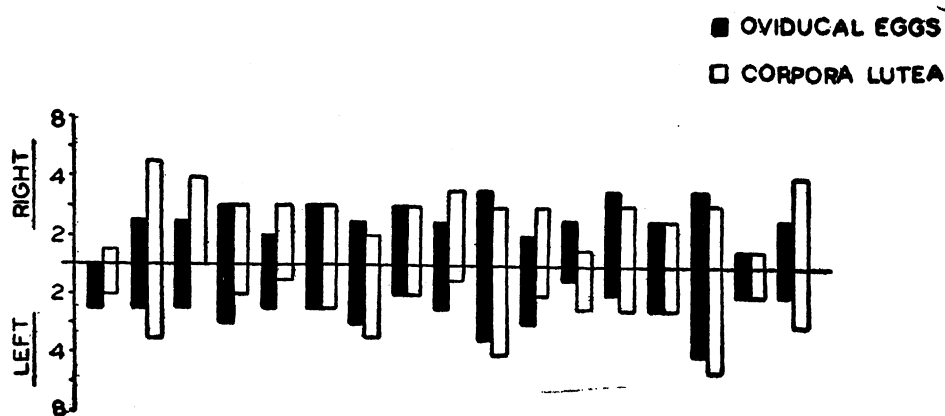


Figure 3. Comparative counts of oviducal eggs and corpora lutea in *Kachuga smithi* (represented by black and white bars, respectively).

differed from one another but corresponded well to the total number of corpora lutea as on their respective sides. One turtle had a balanced number of corpora lutea and oviducal eggs on each side. In the remaining 11 individuals, 5 showed more corpora lutea on the right side whereas the oviducal eggs were more on the left in only two, 3 showing equal number of eggs on both the sides. Out of the remaining six, 5 individuals showed more corpora lutea on left side but the oviducal eggs were more on the left in three, on the right in one and the fifth one had equal number of corpora lutea on the two sides, but the oviducal eggs were more on the right side.

The present studies have thus revealed that in *Lissemys p. punctata*, *Kachuga t. tectum* and *K. smithi*, the phenomenon of transabdominal migration of ova is of a relatively common occurrence. Seventy per cent of the *Lissemys p. punctata* ($N = 10$), 4% of *Kachuga t. tectum* ($N = 15$) and 73% of *K. smithi* ($N = 15$) studied for this phenomenon showed positive evidence of extra-uterine migration of ova. There is no previous record of such a high percentage of transfer as has been recorded presently in *Lissemys p. punctata* and *Kachuga smithi*. The previous highest report of ovular migration (66.6%) has been recorded in *Trionyx muticus* by Plummer (1977). Although reported in some other turtles as well the magnitude of the phenomenon in all of them is rather low being 57% in *Terrapene ornata*; 13% in *T. cerdina* (Legler 1958) and 57% in *Sternotherus odoratus* (Tinkle 1959).

The present observations reveal that in individuals of smaller size below 250 mm (in plastron length) of *Lissemys p. punctata* (table 1), the transfer of ova is much higher (87.5%) than in the larger individuals (of plastron length above 250 mm) of the species. In *Kachuga smithi* of a plastral length of 210 mm or less, the migration is again higher (83%, table 2) than in its bigger individuals, where the transfer of ova was found to be only 49%. In *Kachuga t. tectum*, on the other hand, the sample size being very small did not provide sufficiently reliable data. Thus our findings run counter to those of Tinkle (1959) who has reported for *Sternotherus odoratus*, that the extent of transabdominal migration of ova is higher in bigger individuals (62%) than in smaller ones (50%). Obviously the phenomenon is unrelated to size or age and could be a mere chance or an outcome of an occasional positional shift of the oviduct or ovaries known in reptiles (Cuellar 1970) during the act of encapsulation of the oocytes.

When viewed from the point of imbalance and differential weight of the ovary, a definite relationship between the weight of the ovary and the oviducal eggs on the same side as that of the ovary is evident. In 10 turtles of *Lissemys p. punctata* (table 1) with unequal number of eggs in the two oviducts, 7 showed lesser number of oviducal eggs on the side of heavier ovary, the other three greater number of oviducal eggs on the side of the heavier ovary. In *Kachuga t. tectum* (table 2) only one of the two individuals, suspected of transabdominal migration, had higher egg count on the side with heavier ovary. Of the 10 *Kachuga smithi* (table 3) turtles with imbalanced number of oviducal eggs, 7 showed a higher egg count in the oviduct on the side on which the ovary was lighter, the remaining 3 showing heavier ovary on the side with more eggs in the oviduct. After pooling the data from the three turtles and subjecting these to X^2 , it is found that the P value stands between 0.05 to 0.20, which make deviation to be a matter of chance

Table 4. Percental values of corpora lutea during the breeding season in the two ovaries of *Lissemys p. punctata*, *Kachuga t. tectum* and *Kachuga smithi*.

Animal	Corpora lutea			
	Total number	Higher number		Equal number on both sides (%)
		Right (%)	Left (%)	
<i>Lissemys p. punctata</i>	14	35.7	35.7	28.5
<i>Kachuga t. tectum</i>	30	26.6	56.6	16.6
<i>Kachuga smithi</i>	17	52.3	35.2	11.5

provided the assumption that the expected distribution of the eggs in the two oviducts would be equal (1:1). Since it is not so, the assumption stands untenable. It is therefore, suggested that the imbalance in number of eggs on the two sides has some significance and may help in achieving a better weight balance by having the greater number of ovulatory follicles on the side opposite the greater number of oviducal eggs, particularly in aquatic vertebrates, as has also been suggested earlier by Tinkle (1959).

A perusal of table 4 indicates that neither left nor right ovary in *Lissemys p. punctata* is consistently more productive than the other, although in the emydid turtles (*Kachuga t. tectum* and *K. smithi*) one of the two ovaries tends to be slightly more productive than the other. But the data in tables 1, 2 and 3 indicate that there is no positive relationship between greater productivity of any one ovary and the migration of the eggs. Present findings, however, do not support Legler's (1958) view that in reptiles, the two ovaries show differential activity in different years, one being more active during one breeding season than the other.

Nevertheless, a definite relationship appears to exist between the heavier ovary and lesser number of eggs on a side, as shown above. Should the asymmetrical position of the stomach in chelones have played any major role in the ovular migration as maintained by Hoddenbach (1966), then transabdominal would have been of a much wider and unfailing occurrence than reported or observed in lizards also in nearly all of which asymmetrical disposition of this stomach has been amply documented (Duda 1965).

In conclusion, therefore, the imbalance in the number of eggs on the two sides could be related in fair probably to the physiological necessity of achieving balance at least in the aquatic forms.

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References

- Arey L B 1954 *Developmental anatomy* (Philadelphia : Saunders) 6th ed. V-XI + 680 pp.
- Asdeil S A 1946 *Patterns of mammalian reproduction* (Ithaca : Comstock) XVI—437 pp.
- Cox W A and Marion K A 1978 Observations on the female reproductive cycle and associated phenomena in spring-dwelling population of *Sternotherus minor* in North Florida (Reptilia : Testudines) ; *Herpetologica* **34** 20-33
- Cuellar O 1970 Egg transport in lizards ; *J. Morphol.* **130** 129-135
- Duda P L 1965 *Functional morphology of Agama tuberculata* Gray. Ph.D. thesis, Kashmir University
- Goldberg S R 1972 Reproduction in the southern alligator lizard *Gerrhonotus multicarinatus* ; *Herpetologica* **28** 267-273
- Hoddenbach G 1966 Reproduction in western Texas, *Cnemidophorus sexlineatus* (Sauria; Teiidae) ; *Copeia* 1966 pp. 110-113
- Legler J M 1958 Extra-uterine migration of ova in turtles ; *Herpetologica* **14** 49-59
- Mayhew W W 1963 Reproduction in the granite spiny lizard, *Sceloporus orcutti* ; *Copeia* 1963 pp. 144-152
- Mayhew W W 1965 Reproduction in the sand dwelling lizard, *Uma inornata* ; *Herpetologica* **21** 39-55
- Mayhew W W 1966 Reproduction in the arenicolous lizard, *Uma notata* ; *Ecology* **47** 9-19
- Mayhew W W 1971 Reproduction in the desert lizard, *Dipsosaurus dorsalis* ; *Herpetologica* **27** 57-77
- Moll E O and Legler J M 1971 The life history of neotropical slider turtle, *Pseudemys scripta* (Schöepff) in Panama ; *Bull. Los. Ang. Country Mus. Nat. Hist. Sci.* **11** 1-102
- Plummer M V 1977 Reproduction and growth in the turtle, *Trionyx muticus* ; *Copeia* 1977 pp. 440-447
- Smith M A 1931 *The Fauna of British India, including Ceylon and Burma* (London : Taylor and Francis) pp. 188
- Telford S R 1969 The ovarian cycle, reproductive potential and structure in a population of the Japanese lacertid *Takydromous tachydromoides* ; *Copeia* 1969 pp. 548-561
- Tinkle D W 1957 Ecology, maturation and reproduction of *Thamnophis sauritus proximus* ; *Ecology* **38** 69-77
- Tinkle D W 1959 Additional remarks on extra-uterine migration of ova in turtles ; *Herpetologica* **15** 161-162
- Tinkle D W 1961 Population structure and reproduction in the lizard *Uta stansburiana steinogeri* ; *Am. Midl. Nat.* **66** 205-234
- Weekes H C 1935 A review of placentation among reptiles with particular regard to the function and evolution of the Placenta ; *Proc. Zool. Soc. London* pp. 625-645
- White J B and Murphy G G 1973 The reproductive cycle and sexual dimorphism of the common snapping turtle, *Chelydra serpentina serpentina* ; *Herpetologica* **29** 240-246

Sediment-polychaete relationship in the Vasishta Godavari estuary

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Abstract. A 16 km stretch of the lower Vasishta Godavari estuary (lat. $16^{\circ} 18' N$ long. $81^{\circ} 42' E$) was surveyed during October 1976-January 1978 to study the polychaete-sediment relationship. Mean high mid- and low water marks at six permanent stations were sampled for studying polychaete distribution as well as sediment characteristics. Sand fraction dominated stations I and II and the silt-clay per cent increased higher up the estuary. Organic matter in the estuary ranged from 0.1 to 4.2% and the amount is generally linked with the silt-clay fraction of the sediment. Depending upon their tolerance to the sediment composition polychaete species colonised different tidal levels. Carnivores were restricted to sandy substrata. For the detritus feeders, the influencing factor appears to be organic matter than the sediment composition.

Keywords. Sediment composition ; organic matter ; relationship ; carnivores ; detritus feeders.

1. Introduction

The importance of the substratum during settlement of polychaete larvae has been documented by Wilson (1953). Sanders (1958) successfully attempted to relate the type of feeding of the organism and the sediment composition in Buzzards Bay, Massachusetts. Thus he found detritus feeders restricted to the mud sediments and filter feeders to the median grain size sediments. In contrast Muus (1967) stated that in any estuary, with irregular or unfavourable fluctuating physical factors, salinity and dissolved oxygen are more important than the sediment composition in influencing the species distribution. Moreover sediment particle size is known to be a function of the mixing and dilution of salt water by freshwater and therefore particle size is dependent on salinity (McNulty *et al* 1962). Muus (1967) therefore concluded that any attempt in that direction is fruitless. However, later works in several other areas revealed the apparent relationship between the substratum and the invertebrate fauna in general and polychaetes in particular.

In the present study an attempt has been made to establish the possible relationship between the abundance of polychaete fauna and the intertidal sediments in the Vasishta Godavari estuary.

2. Area of investigation

The area presently investigated is the intertidal habitat of the Vasishta Godavari estuary, the southernmost branch of the river Godavari, opening into Bay of Bengal at Antervedi (lat. $16^{\circ} 18' N$; long. $81^{\circ} 42' E$). The geographical description of the area and location of the stations have already been given by Srinivasa Rao (1980).

3. Materials and methods

Collections were made from six stations at monthly intervals, from October 1976 to January 1978 excepting in August 1977 due to fast currents associated with high annual floods. At each station sampling was made from three tidal levels viz., mean high water mark (MHWM), mean mid water mark (MMWM) and mean low water mark (MLWM). Sediment was collected using a PVC corer while a metal frame of $20 \times 20 \times 15$ cm dimensions was used for polychaete collection. Techniques employed for the collection and analysis of hydrographic parameters were the same as described earlier (Srinivasa Rao 1980; Srinivasa Rao and Rama Sarma 1980). Sand, silt and clay fractions in the sediment were estimated by the pipette method of Krumbein and Pettijohn (1938) whereas the organic matter in the sediment was estimated by the method of Gaudette *et al* (1974).

4. Results

The nomenclature of Folk (1968) is adopted to classify the sediments of Vasishta Godavari estuary and the sediment composition during different seasons is presented in figure 1. The sediments were generally sandy near the river mouth (stations I and II) as the area is influenced by neretic waters and the silt-clay fraction increased with the increasing distance from the river mouth (stations III to VI). Along the transect, the sediment composition varied with increasing silt-clay content down the transect. Generally the upper 3 cm layer of the substratum is an unconsolidated layer while below that is a closely packed silt-clay fraction. This layering is the cumulative result of depositional and erosional factors operating during the tidal cycles and the superimposed annual freshwater floods.

The maximum, minimum and average organic matter content for all the tidal levels is presented in table 1. The organic matter content is significantly high in the estuary as also observed by Dora and Borreswara Rao (1975). The low organic matter content at the seaward stations (I and II) may be due to the sandy nature of the substratum and sufficient aeration. The increased silt clay fraction and consequent compactness of the sediment and poor aeration resulted in the retention of a high amount of organic matter at stations III to VI. That the clay minerals bind organic matter better than the loose sands is well-known (Sanders 1956). Similar relationship of the organic matter with fine sediments around the Indian subcontinent was observed by Murthy *et al* (1969), Parulekar *et al* (1976) and Ansari *et al* (1977). The high organic matter content in the sediment during summer is the result of high organic production characteristic of the estuaries. Further the contribution from the adjoining mangroves and terrestrial sources is

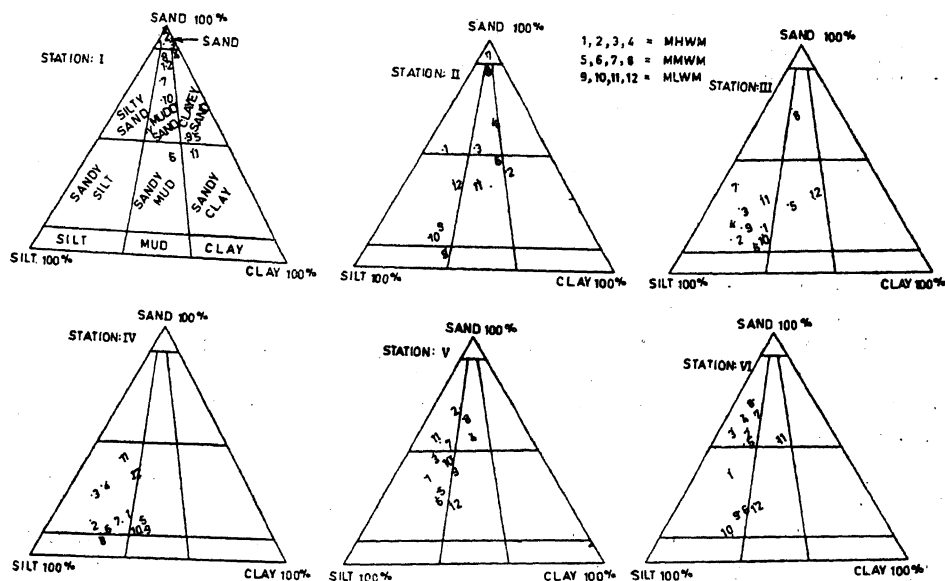


Figure 1. Seasonal variations in the sediment composition during the period of study.

Table 1. Organic matter content in the sediments during the study period.

Station	Tidal level	Minimum (%)	Maximum (%)	Average (%)
I	MHWM	0.11	1.20	0.61
I	MMWM	0.16	2.49	1.53
I	MLWM	1.04	2.64	1.71
II	MHWM	0.17	1.73	0.86
II	MMWM	0.66	3.20	1.85
II	MLWM	0.94	3.52	1.81
III	MHWM	0.39	2.64	1.98
III	MMWM	0.62	3.66	2.25
III	MLWM	0.72	3.20	2.11
IV	MHWM	0.16	3.20	1.99
IV	MMWM	0.97	3.84	2.15
IV	MLWM	0.80	2.62	1.83
V	MHWM	0.27	2.14	1.31
V	MMWM	0.39	2.49	1.73
V	MLWM	0.15	2.57	1.77
VI	MHWM	0.41	4.20	2.34
VI	MMWM	0.38	3.32	2.43
VI	MLWM	0.17	3.74	2.39

remarkably high. The high bacterial activity because of high temperature is yet another factor by which the organic matter reduces in respect of particle size and gets adsorbed onto the sediment.

5. Discussion

In estuaries, the sediment is of paramount importance in influencing the life in general and the benthic fauna in particular. The importance of soil grade as a factor in the distribution of polychaetes has long been recognised (Day and Wilson 1934 ; Southward 1957 ; Bassindale and Clark 1960 ; Clark and Haderlie 1960, 1962 ; Bloom *et al* 1962 ; Williams 1962 ; Estcourt 1967 ; Nichols 1970 ; Boyden and Little 1973 ; Wolff 1973 ; Gray 1974 ; Santos and Simon 1974 ; Grassle and Grassle 1974 ; Buchanan 1963 ; Vietez 1976 ; Whitlatch 1977 and Amaral 1979). Though the food and feeding habits of the polychaetes inhabiting this estuary have not been worked out, the investigations of Sanders (1956) ; McNulty *et al* (1962) and Brett (1963) show that a close relationship prevails between the feeding habits of the infauna, gross organic matter content and the texture of the sediment. Observations made on the morphological features of the polychaetes of this estuary suggested that majority of them are detritus feeders. This is due to the excessive silt-clay fractions in the sediments. The filter feeders are absent up in the estuary as they need well aerated substrata. Such substratum is available at MHW at station I but prolonged period of exposure and high temperature may be acting as deterrent factors preventing their settlement.

Depending upon their tolerance to the substratum composition, different species occupied different positions along the transect, however in varying numbers. The capitellid *Heteromastus similis*, nephtyd *Nephtys oligobranchia* and nereid *Dendronereis arborifera* appear to have great resistance for exposure, grain size and salinity. They were represented equally at all the three tidal levels (figure 2) and in almost all substrata except in places where the sand content was less than 10% (Rama Sarma and Srinivasa Rao 1980 ; Srinivasa Rao 1980 and Srinivasa Rao and Rama Sarma 1980). Several other species which cannot tolerate hard substrata at MHW restricted themselves to the lower tidal levels (figure 2) where the sediment composition was suitable (figure 3). But when forced by wave action they survived there for certain periods.

It is interesting to note that though most species exhibited substratum preference, individuals of each species appeared at times in substrata with different composition (figure 3). It may be because of the influence of the sediment in controlling the abundance of the organisms but not their distribution (Holme 1949 ; Wilson 1953 ; George 1964 ; Sanders *et al* 1965). It also appears that mud dwelling species were able to invade the substrata containing sand while the species inhabiting sandy substrata failed to invade the muddy ones. This may be because of the possible clogging of the feeding apparatus of the sandy inhabitants when they try to invade the muddy sediments. Similar observation was made by Johnson (1971). Thus members of the genus *Glycera*, eunicids *Diopatra neapolitana* and *Lumbrineris heteropoda* which are known carnivores, restricted themselves to the sandy substrata. They feed on the interstitial microfauna available in that habitat. For several sedentarian species like *Magelona cincta*, *Cossura coasta*, *Sternaspis*

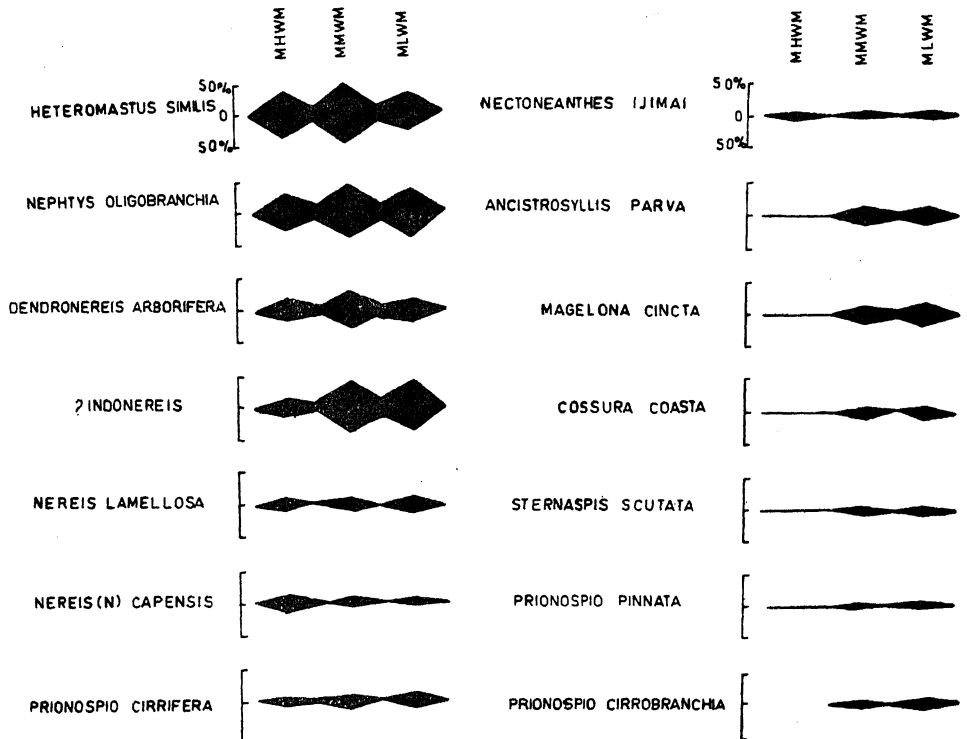


Figure 2. Transectwise distribution of polychaete species in the Vasishta Godavari estuary.

scutata and members of the family Spionidae which are detritus feeders, there appears to be no substratum preference, except the avoidance of sandy substrata.

Nereis lamellosa occurred in good numbers at station I where grass was present which not only provides stability to the substratum but also keeps the temperature low, in addition to providing food material in the form of detritus. The importance of grass in the distribution and food patterns of intertidal organisms was shown by MacGinitie as early as in 1939.

Organic matter in the sediment plays an important role in the abundance and distribution of benthic polychaetes especially in estuaries where the organic matter content available in the shallow water sediments is usually very high. Buchanan (1963) observed that the distribution of the organisms is generally related to the temperature, salinity and grade of the soil and more closely with the organic matter. However the organic matter in the utilisable form in the sediment is reported to be important for the polychaete survival.

Organic matter in the Vasishta Godavari estuary is chiefly of plant origin which is brought down by a multitude of small creeks, finally getting embedded in intertidal sediments. This material is played up and down the estuary and also between MHWM and MLWM in the intertidal region. In addition the intertidal organisms themselves contribute to the organic matter content apart from the minute fraction swept in from the sea through tidal action.

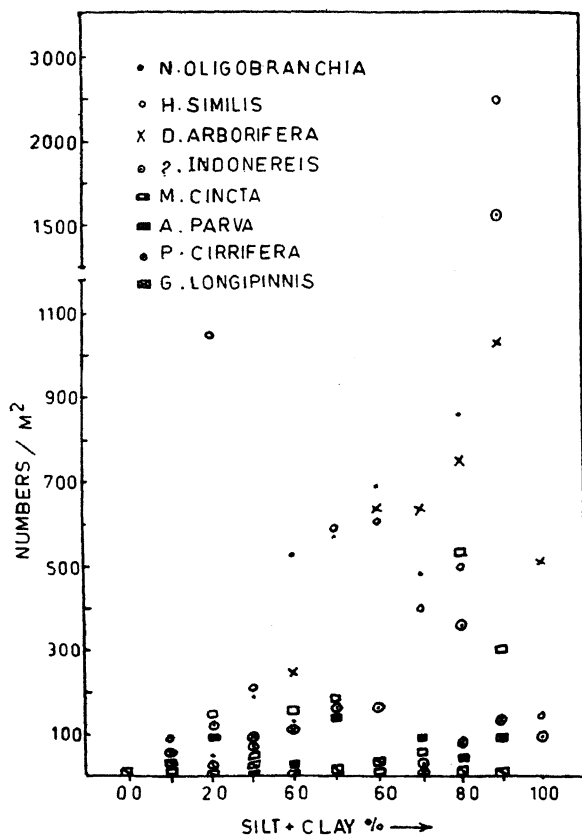


Figure 3. Sediment composition and the density of polychaete species.

The capitellid *Heteromastus similis* was found to be cosmopolitan in distribution (in respect of the nature of substratum) but the major factor which outweighed all other factors is decidedly the organic matter content (Srinivasa Rao 1980). The detritus feeders *Dendronereis arborifera*, *Magelona cincta*, *Sternaspis scutata* and *Cossura coasta* were found in greater abundance in muddy areas where the organic matter content was high. On the other hand the carnivores of the family Glyceridae and Eteimnidae have shown no special relationship with the organic matter. Similar is the case with *Nephtys oligobranchia*, a proven carnivore (Srinivasa Rao and Rama Sarma 1978).

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References

- Amaral A C Z 1979 The ecology and contribution of polychaetous annelids to the benthic biomass of the tidal zones in the northern coast of Sao Paulo state ; *Biol. Inst. Oceanogr. Sao Paulo* **28** 1-52
- Ansari Z A, Harkantra S N, Nair S A and Parulekar A H 1977 Benthos of the Bay of Bengal; A preliminary account ; *Mahasagar* **10** 55-60
- Bassiandale R and Clark R B 1960 The Gann flat dale : Studies on the ecology of a muddy beach ; *Field Studies* **1** 1-22
- Bloom S A, Simon J L and Hunter V D 1972 Animal sediment relationship and community analysis of a Florida estuary ; *Mar. Biol.* **13** 43-56
- Boyden C R and Little C 1973 Faunal distribution in soft sediments of the Severn estuary ; *Esruar Coast Mar. Sci.* **1** 203-223
- *Brett E C 1963 Relationship between marine invertebrate infaunal distribution and sediment type distribution in Bogue Sound, North Carolina. Ph. D. thesis, University of North Carolina
- Buchanan J B 1963 Bottom fauna communities and their sediment relationships off the coast of Northumberland ; *Oikos* **14** 154-175
- Clark R B and Haderlie E C 1960 The distribution of *N. cirrosa* and *N. hombergi* on the South Western coasts of England and Wales ; *J. Anim. Ecol.* **29** 117-147
- Clark R B and Haderlie E C 1962 The distribution of *N. californiensis* and *N. caecoides* on the Californian coast ; *J. Anim. Ecol.* **31** 339-357
- Day J H and Wilson D P 1934 On the relation of the substratum to the metamorphosis of *Scolecopsis fulliginosa* (Claparede) ; *J. Mar. Biol. Assn. U.K.* **19** 655-662
- Dora Y L and Borreswara Rao C 1975 Oragnic matter content of the Vasishta Godavari river sediments ; *Bull Dept. Mar. Sci-Univ. Cochin* **7** 945-952
- Estcourt I N 1967 Ecology of benthic polychaetes in the Heathcote estuary, New Zealand ; *New Zealand J. Mar. Freshwater Res.* **1** 371-394
- Folk R L 1968 *Petrology of sedimentary rocks*. (Texas : Hemphill's Austin) pp 170.
- Gaudette H E, Wilson R F, Toner R and David W F 1974 An inexpensive titration method for the determination of organic carbon in recent sediments ; *J. Sed. Petr.* **44** 249-253
- George J D 1964 On some environmental factors effecting the distribution of *Cirroformia tentaculata* (Polychaeta) at Hable ; *J. Mar. Biol. Assn. U.K.* **44** 373-388
- Grassle J F and Grassle J P 1974 Opportunistic life histories and genetic systems in marine benthic polychaetes ; *J. Mar. Res.* **32** 253-284
- Gray J S 1974 Animal sediment relationships ; *Oceanogr. Mar. Biol. Ann. Rev.* **12** 223-261
- Holme N A 1949 The fauna of sand and mud banks near the mouth of the Exe estuary ; *J. Mar. Biol. Assn. U. K.* **28** 189-237
- Johnson R G 1971 Animal sediment relations in shallow water benthic communities ; *Mar. Geol.* **11** 93-104
- Krumbein W C and Pettijohn F J 1938 *Manual of sedimentary petrology* (New York : Appleton Century Crofts) pp. 549
- MacGinitie G E 1939 Ecological aspects of California marine estuary ; *Am. Midl. Nat.* **16** 629-765
- McNulty J K, Work R C and Moore H B 1962 Some relationships between the infauna of the level bottom and the sediments in South Florida ; *Bull. Mar. Biol. Gulf Carib.* **12** 322-332
- Murthy P S N, Reddy C V G and Varadachari V V R 1969 Distribution of organic matter in the marine sediments off the west coast of India ; *Proc. Nat. Inst. Sci. India* **35** 377-384
- Muus B J 1967 The fauna of Danish estuaries and lagoons : distribution and ecology of dominating species in the shallow water mesohaline zone ; *Medd. Danm. Fisk. Havunders N S* **5** 147-174

* Not referred to in original.

- Nichols F H 1970 Benthic polychaete assemblages and their relationship to the sediment in Port Madison, Washington ; *Mar. Biol.* 6 48-57
- Parulekar A H, Nair S A, Harkantra S N and Ansari Z A 1976 Some quantitative studies of benthos off Bombay ; *Mahasagar* 9 51-56
- Rama Sarma D V and Srinivasa Rao D 1980 Ecology of *Dendronereis arborifera* (Polychaeta: Nereidae) in the Vasishta Godavari estuary. Paper presented at V All India Congr. Zool. Nov. 1980, Bhopal
- Sanders H L 1956 Oceanography of Long Island Sound 1952-1954 Biology of marine bottom communities ; *Bull. Bingham. Oceanogr. Coll.* 15 345-414
- Sanders H L 1958 Benthic studies in Buzzards Bay. 1. Animal sediment relationships ; *Limnol. Oceanogr.* 3 245-258
- Sanders H L, Mangelsdorf Jr. and Hampson G R 1965 Salinity and faunal distribution in the Pocasset river, Massachusetts ; *Limnol. Oceanogr.* 10 216-228
- Santos S L and Simon J L 1974 Distribution and abundance of the polychaetous annelids in a South Florida estuary ; *Bull. Mar. Sci.* 24 669-689
- Southward E C 1957 The distribution of polychaeta in offshore deposits in the Irish Sea ; *J. Mar. Biol. Assn. U.K.* 36 49-75
- Srinivasa Rao D 1980 Ecology of *Heteromastus similis* Southern 1921 (Polychaeta : Capitellidae) in the Vasishta Godavari estuary ; *Proc. Indian Acad. Sci. (Anim. Sci.)* 89 407-414
- Srinivasa Rao D and Rama Sarma D V 1978 Food and feeding habits of *Nephtys oligobranchia* Southern (Annelida : Polychaeta) ; *Indian J. Mar. Sci.* 7 193-195
- Srinivasa Rao D and Rama Sarma D V 1980 Ecology of *Nephtys oligobranchia* Southern from the Vasishta Godavari estuary ; *Indian J. Mar. Sci.* 9 218-221
- Vieitez J M 1976 Ecología de Poliquetos y Moluscos de la playa de Meira (Ria de Vigo) 1. Estudio de las comunidades ; *Inv. Pesq.* 40 223-248
- Whitlatch R B 1977 Seasonal changes in the community structure of the macrobenthos inhabiting the intertidal sand and mud flats of Barnstable Harbor, Massachusetts ; *Biol. Bull. Mar. Biol. Lab. Woodshole* 152 275-294
- Williams G B 1962 The effects of extracts of *Fucus serratus* in promoting the settlement of larvae of *Spirorbis borealis* (Polychaeta) ; *J. Mar. Biol. Assn. U.K.* 44 397-414
- Wilson D P 1953 The settlement of *Ophelia bicornis* Savigny larvae. The 1951 experiments ; *J. Mar. Biol. Assn. U.K.* 31 413-438
- Wolff W J 1973 The estuary as a habitat, an analysis of the data on the soft bottom macrofauna of the estuarine areas of the rivers Rhine, Meuse and Scheldt ; *Zool. Verhandelingen* 126 1-242

The form-function relationship of vertebrates : A selected review

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Abstract. A selected literature dealing with the relationship between vertebrate structures and functions has been reviewed. Published literature in this field generally relates to three approaches: evolutionary, ontogenetic and holistic. This paper explains the salient features of these approaches and how their findings can be verified experimentally. Evolutionary approach can only make use of theoretical explanation, whereas, in both ontogenetic and holistic approaches experimentation is possible.

Keywords. Vertebrates; form-function relationship; evolutionary approach; ontogenetic approach; holistic approach.

1. Introduction

The study of the relationship between vertebrate structures and functions, also referred to as "form-function relationship analysis", is becoming more a part of morphological research than gross anatomy. Published literature in this field generally adheres to three approaches: evolutionary, ontogenetic and holistic. The purpose of this paper is to review selected literature, that explains the important aspects of those approaches and how their findings can be verified experimentally.

1.1. *Evolutionary approach*

Evolutionary approach to the form-function relationship is not a new one. Böker (1935, 1936) establishes that form is derived from function; thus accordingly, function always precedes form. Consequently, he defines that the aim of research should be to describe the functional series, and that along with the phylogenetic development of function concurrent development of form occurs. But, Böker's views are not comprehensive because he considers only the functional aspects of form while neglecting the influence of genetics and convergence. Most of the functional anatomists started as evolutionists (Eaton 1935; Hofer 1948; Gans 1952, 1960; Bock 1959, 1964; Davis 1949, 1958, 1964; Gutmann 1966, 1967, 1968). Their research is mainly based on the shape or structure of living organisms. They consider the function or change in the function as parameter of the

structure, therefore, any change in the structure causes parallel development in the function or evolution.

Bock and DeWitt (1959), in a study on the position of the toes in birds in relation to their locomotion, distinguished six types of toes which perform two functions, climbing and perching. Those six types of toes are all irregularly distributed over the taxonomic groups. Bock and DeWitt are of the opinion that the various types of toes have developed under the influence of selective forces of function (functional requirement). Bock (1960) considers the supplementary joint between the lower jaw and the cranium to be a pre-adapted structure which is a bracing mechanism that withstands the strong force exerted on the lower jaw during prey-catching. Gans (1952, 1960, 1966) has given similar evolutionary approach to functional anatomy. According to him a general body plan is formed genetically, upon which the functional influences are logically superimposed to develop the modified structures. Another evolutionary approach considered by Liem (1967a, b, 1970) combines comparative and deductive methods. Greenwood (1965) has correlated the environmental effects on the pharyngeal gills of cichlid fish. His work researches the adaptive strategies in the pharyngeal jaws based on the effects of the natural environment. Additionally, mosaic evolutionary approach has been postulated by DeBeer (1954) in which he indicates that the transitional changes do not involve a single organ-system but are rather functionally integrated structural complexes. For example, modifications in the feeding mechanism generally include changes in the skull, jaw musculature, and circulation. In describing the evolution of bony fish, several authors have emphasized the specializations and adaptations of the skull (jaws) and the muscles. The bony fish tend to optimize these structures of the head for food intake (Schaeffer and Rosen 1961).

1.2. *Ontogenetic approach*

Several functional morphologists have interrelated the developing elements at specific ontogenetical stages. Even in their developing stages, the elements are integrated in a pattern by their properties. These properties have been subdivided into coherence, presence, position, size and shape (Dullemeijer 1974).

It has been suggested that the individual parts of living organisms must develop in coherence with each other. To illustrate this, Milaire (1963) and Landsmeer (1968) have established a coherent system of phalangi with their surrounding tendons and muscles in a developing hand. Accordingly, the parts are arranged in specific positions in a limited space. The specific position and spatial coherence are needed for the parts to realize their function.

The simultaneous occurrence of several elements or organs seems to fulfil the functional demand of a growing organism. The functional interdependence of two specific elements like muscles and jaws in the amphibian larvae has been confirmed by Gaupp (1905), Sedrá (1950) and DeJongh (1968).

Elements develop as a result of the differentiation of homogeneous materials into heterogeneous structures. Heterogeneity of structures evolves at different stages of development. Wolpert (1968) has suggested the process by which a heterogeneous area evolves from a homogeneous one.

Elements may be divided into dominant and subordinate sub-groups. The dominant elements have a strong influence in early development. In the head the

central nervous system seems to be dominant in all stages and is followed by the sense organs and the pharyngeal cavity (Dullemeijer 1971). Based on the dominance of surrounding mesenchymal tissue in the formation of mouth and the middle ear cavities, Goedbloed (1964) suggested that the formation of those cavities is controlled by the shifting of the epithelial border in relation to the mesenchyme. Thus, mesenchyme seems to be the dominant structure in the development of mouth and middle ear cavities. However, an opposite viewpoint has been put forth by Moss (1971), who suggests that oral and middle ear cavities influence the formation of mesenchyme. Moreover, the importance of the presence of the surrounding elements has been observed by Blechschmidt (1955) in the descent of a male gonad. Thus, a certain morphological arrangement is essential for a male gonad to descend.

The position of the elements in the process of ontogeny is also significant to their functions. Werner (1958, 1959) states that the position of the elements shifts greatly during ontogeny in order to carry out their activities. The specific position for the specific element is essential to carry out certain functions in a particular spatial arrangement (Landsmeer 1968). Therefore, position of the growing elements is related to their functions. As some elements are dominant they influence the position, form and structure of other elements. Such influences have been indicated by DeJongh (1968) and Moss and Salentijn (1969a, b). Moss (1968c) suggests that the positions of many growing skeletal elements are passively displaced because of the growth of other elements. Positional changes of the elements influence the determination of the general body plan (Moss and Young 1960; Moss and Salentijn 1969b).

Size also influences the process of development. Balinsky (1965) establishes a correlation between the amount of yolk and the process of gastrulation. The size or the amount of yolk determines the process of cleavage and gastrulation. The change in the size of an element such as a muscle will have a functional effect (exertion) on the other element (e.g., on a bone). Moreover, the change in the size will have impact on the shape of the local elements (Dullemeijer 1974).

The shape of the skull changes under the influence of muscle attachments and the weight it carries. The skull of pigs and elephants may be cited as examples (Dullemeijer 1974). The shape of a developing jaw is influenced by the size of the muscle attached to it. Moss and Salentijn (1969b) indicate that the general shape and position of an element depends on the position and size of other elements (e.g., the position of the jaws depends on the oral cavity and the position of the calvaria bones depends on the size and shape of the growing brain).

A switchover in the properties of an element has been observed by Claes (1964, 1965). He suggests that the chorda carries out the inductive function at an early stage but the same structure transforms into a supporting bar at a later stage. Such structural and functional switchovers in the elements are not infrequent. For example, during the endochondral ossification the cartilaginous structure is transformed into a bone.

Thus, during the process of ontogeny one can observe a spatially coherent system and interdependence of the developing elements with respect to their position, size and shape. The most important requirement of the elements is to carry out their functional demands.

1.3. Holistic approach

Recently, the functional anatomists have applied a holistic principle to the functional analysis of form. The holistic principle in its most modern form has been initiated by Van der Klaauw (1945, 1948, 1951, 1952). He was the first to introduce the concept of holism in modern morphology and was followed by Moss (1958, 1959, 1960, 1961b, 1968a, b), Dullemeijer (1956, 1958, 1959, 1974), Goss (1964), Dutta (1968, 1975, 1979a, b, 1980), and Osse (1969). There is, however, a difference of opinion amongst the functional morphologists regarding holism in relation to form and function. For example, Russell (1936), Smit (1961) and Goss (1964) believe that the specific structures develop after the influence of the function, while Bock (1959) postulates that function is caused by structures. On the other hand, Rensch (1948, 1958, 1960, 1972) formulates that there may have been structures without a function and, in turn, the non-functional structures may acquire new functions. He believes that a causal relationship exists between function and form though most other modern functional anatomists reject such a relationship (Barge 1919, 1936).

In order to correlate form and function, the head has been considered to be composed of several functional components which form a totality (Dutta 1975). The "functional component" has been described by Van der Klaauw (1948, 1951, 1952) and Dullemeijer (1956, 1958, 1959, 1971) as a morphological structure of an element which performs a certain function. According to Klaauw (1945), Dullemeijer (1956) and Dutta (1968, 1975) the components have a well-defined individuality which is determined by the components themselves and by the pattern of the skull. The components of the head are in turn composed of closely related elements such as bony elements, ligaments, muscles and other tissues which perform one or more functions together as mentioned by Bock (1964), Liem (1967a, b) and Dutta (1968, 1975, 1979, 1980). These functional units (elements) are connected and form couplings (Liem 1967a, b; Dutta 1975) which conduct the function of the animals.

Dutta (1975) has illustrated two such couplings in *Anabas testudineus* and *Ctenopoma acutirostre*. They are: (a) the levator operculi-opercular apparatus-mandibular coupling (regarded to cause depression of the lower jaw during fish respiration) and (b) the sternohyoideus-hyoid apparatus-interopercular-mandibular coupling (which collaborates with the former coupling during feeding).

A functional component cannot maintain its separate entity because in order to carry out its functions, it becomes involved with the elements of its neighbouring component(s) (Dutta 1975). This was further illustrated by Moss and Young (1960) who have conceived that the maxilla, which forms the orbit, is somehow related to vision while it also relates to the function of biting along the palatine. The interdependence of functional units is also emphasized by Gans (1969) when he states that, "The structures tend to be affected by the influence of multiple functions and any function will almost certainly affect multiple characteristics of an animal." This overlap between two or more functional components is not only limited to function, but also to structure as well as space.

2. Experimental analysis

Based on the philosophy of form-function relationship, several scholars have studied anatomy since the turn of the century. As early as 1903, Allis worked on the functional aspects of the skull, cranial, first spinal muscles, and nerves in *Scomber scomber*. Takahasi (1925), Tchernavin (1953), Holmquist (1910) and Edgeworth (1935) have also investigated functional aspects of structures. Their interpretations and conclusions were based on anatomy and visual observations. A new approach to the form-function relationship was established by Klaauw (1945, 1963) and followed by Dullemeijer (1974), Gans (1969), Liem (1967a, b), Barel *et al* (1976), Young (1969), Osse (1969), Sarkar (1960), Dutta (1968, 1973, 1974, 1975, 1977, 1978, 1979a, b, 1980). Elshoud-Oldenhavé and Osse (1976), Lauder (1979, 1980a,b), and Lauder and Liem (1980). This philosophy in turn has become more apt to empirical experimentation with the introduction of electromyographic techniques and high-speed cinematography.

Within the last one and a half decades functional anatomists have begun to analyse experimentally the feeding and respiratory mechanisms of vertebrates. These experimental studies involve high-speed cinematography (Dutta 1968, 1975, 1979, 1980; Liem 1967b, 1970; Lauder 1979; Nyberg 1971).

It is well known that in many families of the bony fish, food is obtained by sucking (Alexander 1970). These movements are very fast (20–50 m second) and negative pressures from 100 to 400 cm of water have been registered (Hughes 1970). Therefore, a high speed, movie camera of 500–1000 frames/second is essential to make a precise recording of movements of the bony elements as well as the entire mechanism of prey intake of fish and other vertebrates. Food intake is the dominating function in fish populations and depends on the rapidness of movements of bony elements as well as the activity of their related muscles.

Synchronized electromyographic (EMG) and cinematographic techniques have been adopted by Ballintijn *et al* (1972), Elshoud-Oldenhavé and Osse (1976), Lauder and Liem (1980a, b), Liem (1973, 1978), Liem and Osse (1975) and Osse (1969). Some authors have also used the cinematographic-electromyographic technique in higher forms of vertebrates (Kallen and Gans 1972; Weijs and Dantuma 1975). The usual cinematographic technique has been improved through the use of x-ray movies (Anker *et al* 1967). The x-ray cinematography is being extensively used by the researchers in Anglo-America as well as in Europe for the analysis of bone movements.

3. Conclusions

To establish form-function relationship all three approaches (evolutionary, ontogenetic and holistic) may be considered scientific, but there are relative advantages and disadvantages in their experimentation. Since verification of evolutionary findings is experimentally impossible, this approach deals with theoretical explanation. As different stages of development of an organism (including very minute, embryonic structures) are involved in ontogenetic studies, microscopic analysis, in addition to the application of electromyographic and cinematographic techniques, is necessary wherever required. Nonetheless, electromyographic technique is almost impossible to apply in small size specimens at the early stages of verte-

brate development. However, since the holistic approach normally involves mature organisms it is generally possible to apply all three techniques (microscopic, electromyographic and cinematographic) without much difficulty.

References

- Alexander R Men 1970 Mechanics of the feeding action of various teleost fishes ; *J. Zool.* **162** 145-156
- Allis E P 1903 The skull and cranial and first spinal muscles and nerves in *Scomber scomber* ; *J. Morphol.* **18** 45-328
- Anker G C, Simons J and Dullemeijer P 1967 An apparatus for direct x-ray cinematography exemplified by analysis of some respiratory movements in *Gasterosteus aculeatus* ; *Experimentia* **23** 74-77
- Balinsky B I 1965 *An introduction to embryology* (Philadelphia : Sanders) p. 725
- Ballintijn C M, Burg A Van Der and EG-Erink B P 1972 An electromyographic study of the adductor mandibulae complex of a free swimming carp (*Cyprinus carpio*) during feeding ; *J. Exp. Biol.* **57** 261-283
- Barel C D N, Witte F and Vanooijen M J P 1976 The shape of the skeletal elements in the head of a generalized Haplochromis species : *H. elegans trewavas.* 1933 (Pisces, Cichlidae) ; *Neth. J. Zool.* **26** 163-265
- Barge J A J 1919 *Vorm en functie* (Leiden : Rede Publishers) p. 29
- Barge J A J 1936 *Forme et fonction. La nature du probleme* ; *Folia Biotheoretica* **1** 12-26
- Blechschildt E 1955 Embryologische untersuchungen unter funktionellen gesichtspunkten ; *Acta Anat.* **24** 339-392
- Bock W J 1959 Preadaptation and multiple evolutionary pathways ; *Evolution* **13** 194-211
- Bock W J 1960 Secondary articulation of the avian mandible ; *Auk* **77** 19-55
- Bock W J 1964 Kinetics of the Avian Skull ; *J. Morphol.* **114** 1-42
- Bock W J and DeWitt M 1959 The scansorial foot of the Woodpeckers with comments on the evolution of perching and climbing feet in birds ; *Am. Mus. Novit.* **1931** 45
- Böcker H 1935 Vergleichende biologische Anatomie der Wirbeltiere ; *I* (Jena: Gustav Fisher) p. 228
- Böcker H 1936 Form und Funktion im Lichte der vergleichenden biologischen Anatomie ; *Folia Biotheoretica* **1** 27-41
- Claes H 1964 Das volumenwachstum des körpers, seiner blasteme and organe während den embryonalund frühen Larvalentwicklung von *Rana temporaria* ; *Acta Anat.* **59** 229-281
- Claes H 1965 Histogische differenzierung Anderung des volumenverteilungsmusters und morphogenetische bedeutung der chorda dorsalis Während den Embryonal und Larvalentwicklung von *Rana temporaria* L. ; *Acta Anat.* **62** 104-156
- Davis D D 1949 Comparative anatomy and the evolution of vertebrates ; *Genetics Paleontol. Evolution* pp. 64-89
- Davis D D 1958 Tarsal ligaments of the spectacled bear, *Tremarctos ornatus* ; *Fieldiana, Zool. Mem.* **39** 19-105
- Davis D D 1964 The Giant Panda ; *Fieldiana Zool. Mem.* **3** 339
- DeBeer G R 1954 Archaeopteryx and evolution ; *Adv. Sci.* **42** 1-11
- DeJongh H J 1968 *Functional morphology of the jaw apparatus of larval and metamorphosing Rana temporaria.* L. (Leiden : E J Brill) p. 103
- Dullemeijer P 1956 The functional morphology of the head of the common vipera *Vipera berus* (L.) ; *Arch. Neerl. Zool.* **11** 386-497
- Dullemeijer P 1958 The mutual structural influence of the elements in a pattern ; *Arch. Neerl. Zool.* **13 Suppl.** pp. 174-188

- Dullemeijer P 1959 A comparative functional-anatomical study of the heads of some Viperidae ; *Morphol. Jahrb.* **99** 881-985
- Dullemeijer P 1971 Comparative ontogeny and cranio-facial growth. In : *Cranio-facial growth in man* (eds.) R E Moyers and W Krogman (Oxford ; Pergamon Press) pp. 45-75
- Dullemeijer P 1974 *Concepts and approaches in animal morphology*. 1-120. vanGorcum, Assen. The Netherlands, p. 264
- Dutta H M 1968 *Functional morphology of the head of Anabas testudineus* (Bloch) (Leiden, Netherlands : Drips Repro. N. V. Meppel), p. 146
- Dutta H M 1973 Functional correlation between the structure of the head and feeding mechanisms in *Ctenopoma acutirostre* ; *Anat. Rec.* **175** 310
- Dutta H M 1974 Structural analysis of the hyomandibula in *Macropodus opercularis*, *Ctenopoma acutirostre*, and *Anabas testudineus* ; *Anat. Rec.* **178** 348-349
- Dutta H M 1975 The suspensorium of *Ctenopoma acutirostre* : A comparative functional analysis with *Anabas testudineus* (Bloch) ; *J. Morphol.* **146** 457-478
- Dutta H M 1977 Functional basis of *Micropterus salmoides* : An electromyographic and cinematographic analysis ; *Anat. Rec.* **187** 570-571
- Dutta H M 1978 Feeding mechanism of largemouth bass ; *Am. Zool.* **18** 622
- Dutta H M 1979a An Electromyographic and cinematographic analysis of the feeding mechanism in *Ictarulus melas* (Catfish) ; *Anat. Rec.* **190** 387
- Dutta H M 1979b Form and function of jaws during feeding. *Ctenopoma acutirostre*, *Anabas testudineus* and *Macropodus opercularis* ; *Acta Morphol. Neerl.-Scand.* **17** 119-132
- Dutta H M 1980 Comparative analysis of the hyomandibula during respiration in Anabantoid teleost fishes : *Macropodus opercularis* in relation to *Ctenopoma acutirostre* and *Anabas testudineus* ; *Zoomorphol.* **94** 185-202
- Eaton T H 1935 Evolution of the upper jaw mechanism in teleost fish ; *J. Morphol.* **58** 157-172
- Edgeworth F H 1935 *The cranial muscles of vertebrates* (Cambridge Univ. Press)
- Elshoud-Oldenhave M and Osse W M 1976 Functional morphology of the feeding system in the Ruff—*Gymnocephalus cerna* (L. 1758) (Teleostei, Percidae) ; *J. Morphol.* **150** 399-422
- Gans C 1952 The functional morphology of the egg-eating adaptations in the snake genus *Dasypeltis* ; *Zoologica* **37** 209-245
- Gans C 1960 A taxonomic revision of the Troglodophinae and a functional interpretation of the Amphisbaenid adaptive pattern ; *Bull. Am. Mus. Nat. Hist.* **119** 129-204
- Gans C 1966 Some limitations and approaches to problems in functional anatomy ; *Folia Biotheoretica* **6** 41-50
- Gans C 1969 Functional components versus mechanical units in descriptive morphology ; *J. Morphol.* **128** 365-368
- Gaupp E 1905 Die entwicklung des kopfskelettes. In : *Handbuch der vergleichenden und experimentellen entwicklungslehre der wirbeltiere.* (ed.) Ottertwig II (Jena : G Fisher) 573-890
- Goedbloed J F 1964 The early development of the middle ear and the mouth cavity. A study of the interaction of process in the epithelium and mesenchyme ; *Arch. Biol.* **75** 207-729
- Goss A N 1964 *Adaptive growth* (London, New York : Academic Press) p. 350
- Greenwood P H 1965 Environmental effects on the pharyngeal gill of a cichlid fish, *Astatoreochromis alluaudi*, and their taxonomic implications ; *Proc. Linn. Soc. London* **176** 1-10
- Gutmann W F 1966 Funktionsmorphologische Beiträge zur 'Castraea-Coelomtheorie' ; *Senck. Biol.* **47** 225-250
- Gutmann W F 1967 Die Entstehung des Coeloms und seine phylogenetische Abwandlung in Deuterostomier-Stamm ; *Zool. Anz.* **179** 109-131
- Gutmann W F 1968 Die funktionelle Beziehung von Achsenskelett und Stamm-Muskel-Apparat der fischartigen Vertebraten ; *Senck. Biol.* **49** 265-272
- Hofer H 1948 Untersuchungen über den Bau des Vogelschädels, besonders über den der Spechte und Steiszhühner ; *Zool. Jahrb. Abt. Anat.* **69** 1-158
- Holmquist O 1910 Der Musculus Protractor Hyoidei (Geniohyoideus Auctt.) und der Senkungmechanismus des Unterdiefers bei den Knochenfischen. *Lunds Univ. Ars.*, N. F. Afd. 2, Bd. 6, Nr. 6 1-24
- Hughes G M 1970 Respiration in an air-breathing fish, the climbing perch, *Anabas testudineus* ; *J. Exp. Biol.* **53** 281-298

- Kallen F C and Gans C 1972 Mastication in the little brown bat, *Myotis lucifugus*; *J. Morphol.* **136** 385-420
- Klaauw C J Van Der 1945 Cerebral skull and facial skull. A contribution to the knowlegde of skull structure; *Arch. Neerl. Zool.* **7** 16-37
- Klaauw C J Van Der 1948, 1951, 1952 Size and position of the functional components of the skull. A contribution to the knowledge of the architecture of the skull base on data in the literature; *Arch. Neerl. Zool.* **9** 1-176; 177-368; 369-559
- Klaauw C J Van Der 1963 *Projections, deepenings and undulations of the surface of the skull in relation to the attachment of muscles* (Amsterdam: N. V. Noord Hollandsche Uitgebers Maatschappij) p. 247
- Landsmeer J M P 1968 Les coherences spatiales et l'equilibre spatial dans la region carpienne; *Acta Anat.* **70** Suppl. **54** 1-84
- Lauder G V 1979 Feeding mechanism in primitive teleosts and in the halecomorph fish, *Amia calva*; *J. Zool. London* **187** 543-578
- Lauder G V 1980 The role of the hyoid apparatus in the feeding mechanism of the coelacanth *Latimeria chalumnae*; *Copeia* **1** 1-9
- Lauder G V and Liem K F 1980a *The feeding mechanism and cephalic myology of Salvelinus fontinalis: form, function and evolutionary significance on Charra (Salvelinus)*. (ed.) E K Balon (The Netherlands: Junk Publishers) pp. 365-390
- Lauder G V and Liem K F 1980b Evolution of the feeding mechanism in primitive Actinopterygian fishes: A functional anatomical analysis of *Polypterus lepisosteus* and *Amia*; *J. Morphol.* **163** 283-317
- Liem K F 1967a A morphological study of *Luciocephalus pulcher*, with notes on gular elements in other recent teleosts; *J. Morphol.* **121** 103-133
- Liem K F 1967b Functional morphology of the head of the anabantoid teleost fish, *Helostoma temminckii*; *J. Morphol.* **121** 135-158
- Liem K F 1970 Comparative functional anatomy of the Nandidae (Pisces: Teleostei); *Fieldiana, Zool.* **56** 166
- Liem K F 1973 Evolutionary strategies and morphological innovations: Cichlid pharyngeal jaws; *Syst. Zool.* **22** 425-441
- Liem K F 1978 Modulatory multiplicity in the functional repertoire of the feeding mechanism in Cichlid fishes; *J. Morphol.* **158** 323-360
- Liem K F and Osse J W M 1975 Biological versatility evolution, and food resource exploitation in African Cichlid fishes; *Am. Zool.* **15** 427-454
- Milaire J 1963 Etude morphologique et cytochimique du developement des membres chez la souris et chez la taupe; *Arch. Biol.* **74** 131-317
- Moss M L 1958 Rotations of the cranial components in the growing rat skull and their experimental alteration; *Acta Anat.* **32** 65-86
- Moss M L 1959 *Disorders of the temporomandibular joint* (Philadelphia: Saunders)
- Moss M L 1960 Functional analysis of human mandibular growth; *J. Prosthet. Dent.* **10** 1149-1159
- Moss M L 1961a Rotation of the otic capsule in bipedal rats; *Am. J. Phys. Anthropol.* **19** 301-307
- Moss M L 1968a A theoretical analysis of the functional matrix; *Acta Biotheoretica* **18** 195-202
- Moss M L 1968b Functional cranial analysis of mammalian mandibular ramal morphology; *Acta Anat.* **71** 423-447
- Moss M L 1968c The primacy of functional matrices in orofacial growth; *Trans. Br. Soc. Study Orthod. and Dental Pract.* **19** 65-73
- Moss M L 1971 Ontogenetic aspects of cranio-facial growth. In *Cranio-facial growth in man* (eds.) R E Moyers and W Krogman (Oxford and New York: Pergamon Press) pp. 109-124
- Moss M L and Salentijn L 1969a The primary role of functional matrices in facial growth; *Am. J. Orthod.* **55** 6 556-577
- Moss M L and Salentijn L 1969b The capsular matrix; *Am. J. Orthod.* **56** 474-490
- Moss M L and Young R 1960 A functional approach to craniology; *Am. J. Phys. Anthropol.* **18** 281-292
- Nyberg D 1971 Prey capture in the largemouth bass; *Am. Midl. Nat.* **86** 128-144

- Osse J W M 1969 *Functional Morphology of the head of the perch (Perca fluviatilis L.) ; An electromyographic study* (Leiden : E J Brill) pp. 289-392
- Osse J W M, Elshoud-Oldenhave M and Vanshie B 1972 A new method for insertion for wire electrodes in electromyography ; *Electromyography* **12** 59-62
- Rensch B 1948 Histological changes correlated with evolutionary changes of body size ; *Evolution* **2** 218-230
- Rensch B 1958 Die Abhängigkeit der Struktur und der Leistungen tierischer Gehirne von ihrer Grösse ; *Die Naturwissenschaften* **45** 145-154 ; 175-180
- Rensch B 1960 Trends toward progress of brains and sense organs ; *Cold Spring Harbor Symp. Quant. Biol.* **24** 291-303
- Rensch B 1972 Neuere Probleme der Abstammungslehre. Die transspezifische Evolution. 3e, durch einen Anhang erweiterte Auflage, XI + 468 p. Stuttgart, Ferdinand Enke Verlag
- Russell E S 1936 Form and Function. A historical note ; *Folia Biotheoretica* **1** 1-12
- Sarkar S 1960 A study of the functional morphology of the head of an Indian puffer fish, *Sphaeroides oblongus* (Bloch). Thesis, Leiden, p. 119
- Schaeffer B and Rosen D E 1961 Major adaptive levels in the evolution of the actinopterygian feeding mechanism ; *Am. Zool.* **2** 187-204
- Sedra S N 1950 The metamorphosis of the jaws and their muscles in the Toad, *Bufo regularis* Reuss, correlated with the changes in the animals' feeding habits ; *Proc. Zool. Soc. London* **120** 405-449
- Smit P 1961 Ontogenesis and phylogenesis—their interrelation and their interpretation ; *Acta Biotheoretica* **15** 1-103
- Takahasi N 1925 On the myology of the cranial muscles of the cypriniform fishes ; *J. Morphol.* **40** 1-103
- Tchernavin V V 1953 On the mechanical working of the head in bony fishes ; *Proc. Zool. Soc. London* **118** 129-143
- Weijs W A and Dantuma R 1975 Electromyography and mechanics of mastication in the albino rat ; *J. Morphol.* **146** 1-34
- Werner C L F 1958, 1959 Relative grössze und lage der organe als faktoren der ontogentischen und phylogenetischen formbildung ; *Wiss. Z. Karl Marx Univ. J.* **8** 7-16
- Wolpert L 1968 The French flag problem. In *Towards a theoretical biology 1, Prolegomena*, (ed.) C H Waddington Edinburgh University Press, pp. 125-133

Metabolic rates and quotients in the cichlid fish, *Tilapia mossambica* (Peters) in relation to random activity

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Abstract. Oxygen consumption, carbon dioxide production and $\text{NH}_3\text{-N}$ excretion increased with increase in random (spontaneous) activity in *Tilapia mossambica* in air-saturated water in tests at 30 and 35°C. The random activity change did not affect the RQ which remained near unity at adequate ambient oxygen. But, the AQ decreased with increase in activity at both 30 and 35°C, suggesting that increased protein utilization in quieter fish when adequate ambient oxygen is available. The routine and standard metabolic rates at 35°C are slightly higher than at 30°C, but, in general, the overall metabolic rates and quotients are significantly in close proximity, suggesting that the temperature range (30-35°C) does not seem to cause a marked metabolic difference in *Tilapia mossambica*.

Keywords. Standard metabolic rate ; routine metabolic rate ; respiratory quotient ; ammonia quotient ; random activity ; *Tilapia mossambica*.

Introduction

Energy utilization for the various biological activities of the whole animal can be known only if the values of metabolism truly reflect the standard (basal), activity or other activity-spelt-out (e.g., swimming speed in fish) state of the animal. The influence of random activity on metabolic rates and quotients (Respiratory quotient, $\text{RQ} = \text{volume of CO}_2 \text{ produced} / \text{volume of O}_2 \text{ consumed}$; Ammonia quotient, $\text{AQ} = \text{the volume or mole : mole relation of NH}_3\text{-N excreted to O}_2 \text{ consumed}$) has been extensively studied only in a few fish (Kutty 1968; Peer Mohamed 1974; Kutty and Peer Mohamed 1975). Under aerobic conditions random activity did not appear to have any effect on the RQ of goldfish and brown trout (Kutty 1968), but the AQ might change with random activity (Peer Mohamed 1974; Kutty and Peer Mohamed 1975). Since *Tilapia mossambica* has been subjected to metabolism studies in relation to several factors (Kutty *et al.* 1972; Kutty 1972; Karuppannan 1972; Peer Mohamed and Kutty 1980; Peer Mohamed 1981), there is lack of information on its standard (basal) metabolic rates and quotients. Present observations deal with metabolic rates— O_2 consumption, CO_2 production and $\text{NH}_3\text{-N}$ excretion—and quotients (RQ and AQ) in *T. mossambica* during random activity at high ambient oxygen.

2. Material and methods

Tilapia mossambica (Peters) were collected from freshwater tanks in and around Madurai and acclimated to freshwater at $30 \pm 0.5^\circ\text{C}$ and $35 \pm 0.5^\circ\text{C}$ for at least 15 days before the experiments. The fish were fed *ad lib* with a formulated food: fish muscle, goat liver and wheat hearts *pro rata* 2:1:1 (Karuppannan 1972). Experimental fish were starved for 24 hr (Peer Mohamed and Kutty 1980) and subsequently left in the respirometer overnight with a continuous flow of water. Tests were performed at the temperature of acclimation.

The apparatus used was a modification of Fry's respirometer (Kutty *et al* 1971b) in which simultaneous measurements of metabolic rate and random activity can be made. Decarbonated tap water, adjusted to a pH of 8.2, was used as explained by Kutty *et al* (1971a).

2.1. Experimental procedure

Each experiment consisted of 7-9 runs of 1 hr in duration, when the respirometer remained closed. Water samples for analyses of dissolved oxygen, total carbon dioxide and ammonia were collected just before and after the closure of the respirometer for individual runs. The respirometer was flushed for 30 min with air-saturated decarbonated water between runs so as to bring the ambient oxygen content near air saturation at the beginning of each run. The random activity of the fish was counted by the difference between the initial and final figure of the activity counter, which was noted immediately after each sampling.

2.2. Methods of water analyses

Dissolved oxygen was measured by using the unmodified Winkler technique (American Public Health Association 1955). The sample used for titration was 25 ml.

Total carbon dioxide was estimated by Maros-Schulek technique (Maros *et al* 1961) modified for fish metabolism studies by Kutty *et al* (1971a) was followed. Fifty ml of water sample was used for each determination.

Ammonia was measured by the method of Stroganov (1962) as described by Kutty (1972). Fifty ml of water sample was distilled, the distillate nesslerised and the optical density read in Bausch and Lomb spectrophotometer (Spectronic-20) at a wavelength of $420\ \mu$. Ammonia-free water (American Public Health Association 1955) was used for blank and to prepare the reagents.

3. Results

Plots of routine oxygen consumption, carbon dioxide production, $\text{NH}_3\text{-N}$ excretion, RQ and AQ against random activity of *T. mossambica* (63.4 g, 16.8 cm) acclimated to and tested at 30°C at ambient oxygen concentration near air saturation are shown in figure 1. Similar plots for *T. mossambica* (64.1 g, 17.0 cm) acclimated to and tested at 35°C are given in figure 2. The present plots for *Tilapia* are simple plots through which a single regression line could easily be fitted (table 1). Mean values of routine metabolism and random activity of *T. mossambica* at 30 and 35°C are given in table 2. The standard metabolic

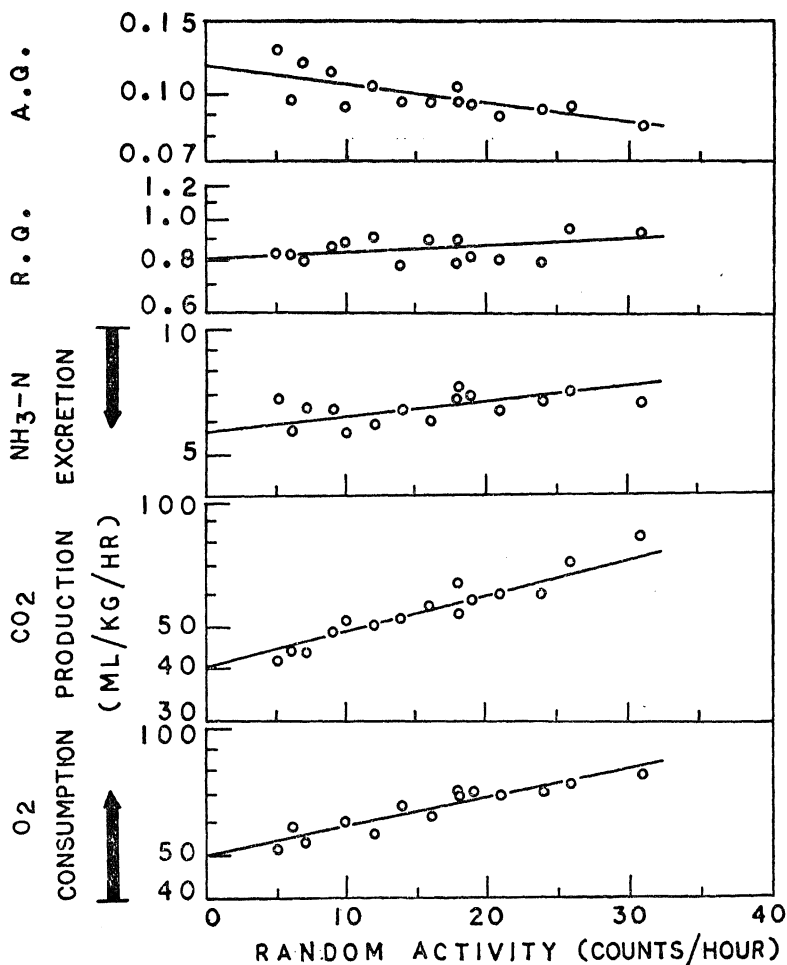


Figure 1. Oxygen consumption, carbon dioxide production, $\text{NH}_3\text{-N}$ excretion, RQ and AQ in relation to random activity in *Tilapia mossambica* acclimated to and tested in air-saturated water at 30°C . The lines fitted through the plots are according to the regression equations given in table 1.

rates and quotients (extrapolated values at 'zero' activity) are also included in table 2.

The routine metabolic rates (O_2 consumption, CO_2 production and $\text{NH}_3\text{-N}$ excretion) at 35°C are slightly higher but the metabolic quotients (RQ and AQ) are remarkably close to each other at 30 and 35°C .

Regression coefficients of metabolic rates and quotients at 30 and 35°C were statistically tested by applying *t*-test and it was observed that the regression coefficients are not significantly different ($P > 0.05$), suggesting that the temperature range ($30\text{--}35^\circ\text{C}$) does not cause a marked metabolic difference in *T. mossambica*.

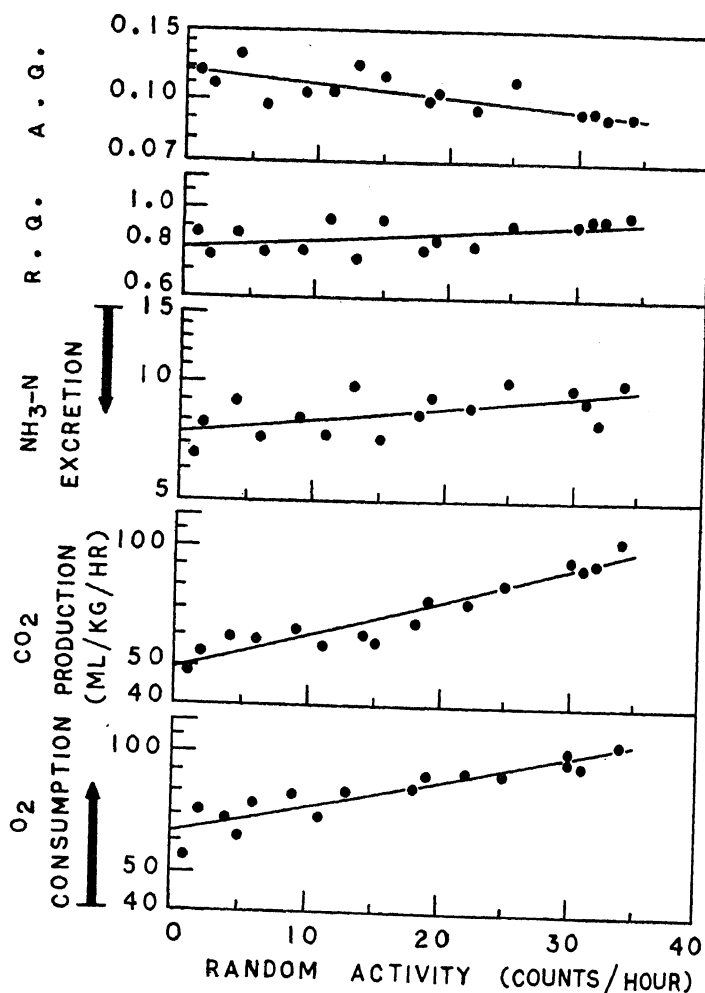


Figure 2. Oxygen consumption, carbon dioxide production, $\text{NH}_3\text{-N}$ excretion, RQ and AQ in relation to random activity in *Tilapia mossambica* acclimated to and tested at 35°C . The lines fitted through the plots are according to the regression equations given in table 1.

4. Discussion

In the results presented (figures 1 and 2), extrapolation of a line drawn through such plots of metabolism against random activity to 'zero' activity indicates the standard metabolic rate of the fish (Kutty *et al* 1971b; Kutty and Peer Mohamed 1975; Peer Mohamed *et al* 1978). The regression lines fitted for plots of O_2 consumption and CO_2 production at 30 and 35°C are almost parallel, suggesting the change in RQ with changes in random activity as observed earlier in the mullet, *Rhinomugil corsula* (Kutty and Peer Mohamed 1975). The slopes of the RQ plots in figures 1 and 2 are positive (table 1). The $\text{NH}_3\text{-N}$ excretion plots show positive trends at 30 and 35°C , but the AQ plots at both temperatures

Table 1. Regression equations ($\log Y = a + bX$) of O_2 consumption, CO_2 production, NH_3 -N excretion (ml/kg/hr in each case), RQ and AQ (Y) on random activity (counts/hr) (X) in *Tilapia mossambica* at ambient oxygen concentration near air saturation.

Acclimation and test at 30° C		Acclimation and test at 35° C	
O_2	$\log Y = 1.69888 + 0.00677 X$	$\log Y = 1.79633 + 0.00644 X$	
CO_2	$\log Y = 1.60628 + 0.00852 X$	$\log Y = 1.68797 + 0.00877 X$	
NH_3 -N	$\log Y = 0.75565 + 0.00342 X$	$\log Y = 0.87070 + 0.00308 X$	
RQ	$\log Y = -0.08831 + 0.00128 X$	$\log Y = -0.09903 + 0.00203 X$	
AQ	$\log Y = -0.92576 - 0.00451 X$	$\log Y = -0.92552 - 0.00335 X$	

Table 2. Routine and standard O_2 consumption, CO_2 production, NH_3 -N excretion (ml/kg/hr in each case), RQ, AQ and random activity (counts/hr) in *Tilapia mossambica* at ambient oxygen concentration near air saturation. Results of experiments at 30 and 35° C are shown separately. In the case of routine values mean and one standard error ($N = 15$ and 16 for 30 and 35° C respectively) is indicated in each case. The standard values are estimates obtained by extrapolation of the regression lines to zero activity through the plots in figures 1 and 2.

Metabolic rate/quotient	30° C		35° C	
	Routine (Mean \pm SE)	Standard	Routine (Mean \pm SE)	Standard
O_2 consumption	64.4 \pm 2.1	50.0	81.8 \pm 3.7	62.6
CO_2 production	55.6 \pm 2.4	40.4	70.6 \pm 4.3	48.7
NH_3 -N excretion	6.5 \pm 0.06	5.7	8.4 \pm 0.27	7.4
RQ	0.86 \pm 0.02	0.82	0.87 \pm 0.02	0.80
AQ	0.101 \pm 0.003	0.119	0.105 \pm 0.003	0.119
Random activity	15.7 \pm 2.0	..	17.0 \pm 2.8	—

yield regressions which have negative slopes (figures 1 and 2, table 1) thereby suggesting that lower random activity was associated with higher AQ, i.e., the less active the fish, the proportionally higher its protein use and/or a greater involvement of protein degradation. This observation is in agreement with earlier reports on *R. corsula* (Kutty and Peer Mohamed 1975) and also on smolting Atlantic salmon (Saunders and Kutty 1973). But there is also a variance in the results of study on the influence of forced activity on AQ which increased with increase in activity (Kutty 1972; Karuppannan 1972; Sukumaran and Kutty 1977). In *T. mossambica*, it was observed that the initial AQ (first hour of 5-hour exercise) at lower swimming speed was even less than the routine AQ (Kutty 1972; Karuppannan 1972) and it was suggested that in this case there might be a

protective action of carbohydrates on protein. This might be the reason why the steady AQ (after 2-3 hours swimming) is correlated well with the initial RQ of fish under long-term exercise. It is possible that random activity (spontaneous random movement) and forced activity (intense exercise) have different relations to protein degradation and utilization, as indicated by $\text{NH}_3\text{-N}$ excretion. A quieter fish would utilize more proteins but with increase in random activity relatively more energy is required by the fish for several breaks and starts in swimming overcoming inertia each time (Smit 1965; Kutty 1969). In this case utilization of carbohydrates could be higher as long as adequate oxygen is available, thereby indicating lower AQ at higher random activity, more or less similar to the low initial AQ during the beginning of intense activity (continued exercise). In both these cases, fish under high random activity and initial phase of intense swimming (forced activity), the protective action of carbohydrates on proteins may be operative (Phillips 1969).

The validity of the present observations lies in the fact that the estimations of metabolic rates and quotients are made in single fish as was usually done in earlier studies (Kutty *et al* 1971b; Peer Mohamed 1974). It is clear from the statistical tests of the regressions that the estimates at 30 and 35°C are remarkably significant, suggesting that the temperature effect is minor. This is possible, probably because these temperatures are close to each other and are within the upper range to which *T. mossambica* is normally exposed during a good portion of the year. The present method described here, which enables separation of the interfering influence of activity on metabolism is of much importance in organismal physiology.

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References

- American Public Health Association 1955 *Standard methods for the examination of water, sewage and industrial wastes* 10th edn. (New York; Am. Publ. Health Org.) pp. 522
- Karuppannan N V 1972 Studies on the locomotory metabolism of *Tilapia mossambica* (Peters); Ph.D. Thesis, Madurai Kamaraj University, Madurai, India
- Kutty M N 1968 Respiratory quotient in goldfish and rainbow trout; *J. Fish. Res. Bd. Can.* **25** 1689-1728
- Kutty M N 1969 Oxygen consumption in the mullet *Liza macrolepis* with special reference to swimming velocity; *Mar. Biol.* (Berlin: Springer-Verlag) **4** 239-242
- Kutty M N 1972 Respiratory quotient and ammonia excretion in *Tilapia mossambica*; *Mar. Biol.* (Berlin: Springer-Verlag) **16** 126-133
- Kutty M N, Karuppannan N V, Narayanan M and Peer Mohamed M 1971a Maros-Schulek technique for measurement of carbon dioxide production in fish and respiratory quotient in *Tilapia mossambica*; *J. Fish. Res. Bd. Can.* **28** 1342-1344
- Kutty M N and Peer Mohamed M 1975 Metabolic adaptations of mullet *Rhinomugil corsula* (Hamilton) with special reference to energy utilization; *Aquaculture* **5** 253-270
- Kutty M N, Peer Mohamed M, Thiagarajan K and Leonard A N 1971b A modification of Fry's fish activity counter and respirometer; *Indian J. Exp. Biol.* **9** 218-222

- Maros L, Schulek E, Molnar-Perl I and Pinter-Szakacs M 1961 Einfaches Destillations verfahren zur titrimetrischen Bestimmung von Kohlendioxyd. (A simple distillation method for the titrimetric determination of carbon dioxide). *Anal. Chim. Acta* **25** 390-399 (Trans. from German, *Fish. Res. Bd. Can. Trans. Ser. No.* 596, 1965)
- Peer Mohamed M 1974 Influence of hypoxia on fish metabolism and activity ; Ph.D. Thesis, Madurai Kamaraj University, Madurai, India
- Peer Mohamed M 1981 Metabolism of *Tilapia mossambica* (Peters) with emphasis on hypoxia ; *Indian J. Exp. Biol.* **19** 1098-1100
- Peer Mohamed M and Kutty M N 1980 Respiratory quotient and ammonia quotient in *Tilapia mossambica* (Peters) with special reference to hypoxia and recovery ; *Hydrobiologia* (In press)
- Peer Mohamed M, Nath D, Srivastava G N and Gupta R A 1978 Influence of sublethal DDT on standard (basal) metabolism of the freshwater fishes *Cirrhina mrigala* (Hamilton), *Labeo rohita* (Hamilton) and *Colisa fasciata* (Bloch and Schneider) ; *Indian J. Exp. Biol.* **16** 385-386
- Phillips A M 1969 Nutrition, digestion and energy utilization; *Fish Physiology* (eds.) W S Hoar and D J Randall (New York and London : Academic Press) **1** 391-432
- Saunders R L and Kutty M N 1973 Oxygen consumption and ammonia excretion in Atlantic salmon smolts (Ms)
- Smit H 1965 Some experiments on the oxygen consumption of goldfish (*Carassius auratus* L.) in relation to swimming speed ; *Can. J. Zool.* **43** 623-633
- Siroganov N S 1962 Methods of study of respiration of fish and methods for ammonia determination, used in studies on fish metabolism ; *Techniques for the investigation of fish physiology* pp. 106-111, (ed.) E N Pavlovskii *Izd. Akad. Nauk SSSR* (Transl. Natn. Sci. Fdn., Washington, D.C., PST Cat. No. 1130 by the Israel Programme for Scientific Translation, Jerusalem, 1964)
- Sukumaran N and Kutty M N 1977 Oxygen consumption and ammonia excretion in the catfish *Mystus armatus*, with special reference to swimming speed and ambient oxygen ; *Proc. Indian Acad. Sci.* **B3** 195-206

Microanatomy of the 7th abdominal ganglion and its peripheral nerves in the scorpion *Heterometrus fulvipes*

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Abstract. Microanatomical studies on the 7th abdominal ganglion of the scorpion were carried out by histological methods. The ganglion revealed a total of approximately 2000 nuclei, most of them belonging to medium-sized cells. The connective and peripheral nerves revealed fibres of varying number in each, with fibres of 3 μm contributing largely to the total content. The fibres included the axons of motor, sensory and interneurons.

Keywords. *Heterometrus fulvipes* ; histological techniques ; microanatomy ; cells ; abdominal ganglion ; peripheral roots.

1. Introduction

Microanatomical knowledge of arthropod abdominal ganglia has been based on highly selective methylene blue stains (Retzius 1890 ; Bethe 1897) and silver-stained serial sections (Kendig 1967). These procedures provided information concerning the course of fibre groups or the location of cell bodies. The available literature on the anatomy of the arachnid central nervous system comes mainly from the studies of Babu (1965). The present investigation attempts to resolve the microanatomy of the 7th abdominal ganglion and its peripheral roots in the scorpion *H. fulvipes* at the light microscopic level.

2. Materials and methods

Freshly collected adult scorpions were anaesthetized with chloroform and carefully dissected under binocular microscope to expose the 7th abdominal ganglion with all its branches intact. The connective tissue around the ganglion and its branches was cleared without any damage to the nervous tissue and the entire preparation was submerged in Bouin's fluid in a Petridish (Babu 1965) for 24 hr. After 24 hr the connective, 4th and 5th segmental and telsonic nerves were cut close to the ganglion and used for sectioning.

The method employed for sectioning was Peterfi's celloidin double embedding method, through which satisfactory results were obtained. After dehydration in methanol series, the material to be sectioned was led through two or three changes of paraffin maintained at 60–62 °C. Use of mixtures of paraffin with different melting points and addition of fresh wax each time before making the block minimized the impediments during sectioning. Cross-sections (10 μm) were taken from the ganglion, connective, 4th and 5th segmental and telsonic nerves. Serial sections of the ganglion were stained by making use of Palmgren's (1948) silver-staining method. The silver method of Holmes (1952) was used for staining the nerve trunks.

After clearing and mounting the sections, the counting of cell number and the measurement of the cell diameter were done as suggested by Abercrombie (1946). The diameter of the nerve fibres was measured under high magnification. The cells and nerve fibres were categorized into different groups based on their diameter, and tabulated according to their size and number.

Preliminary studies to trace the anatomical organization of the 7th ganglion and its nerves were also made using the cobalt chloride back-filling technique (Babu and Subhashini 1981).

3. Results

3.1. *Microanatomy of the 7th abdominal ganglion*

The 7th abdominal ganglion, being double in its nature, consisted of relatively larger number of cells compared to other abdominal ganglia of the cord. In its cross-section (figure 1A) the 7th abdominal ganglion showed the cellular peripheral rind region where the somas of the neurons were located, and a central fibrous neuropile where synapses would occur. The size of the ganglion in its cross-section measured approximately 980–1000 μm wide and 650 μm thick. The cellular rind constituted 1/3 of the total area of the ganglion and the remaining 2/3 was occupied by the neuropile. The ganglion was enclosed in a thick enveloping sheath of 12 μm which was found to be heterogeneous, containing 4–6 layers of tissue which were closely packed. There was no cellular perineurium beneath this neural lamella. Cross-section of the 7th abdominal ganglion revealed the presence of numerous cells, with their nuclei staining dark in colour. They (the nuclei) ranged from 3–20 μm in diameter and they were found to be distributed ventro-laterally and no cells were found on the dorsal side of the ganglion. The nuclear counts were made by applying Abercrombie's formula. The nuclei were distinguished as belonging to three sizes, viz., large, medium and small. The 7th abdominal ganglion comprised of a total of approximately 2033 nuclei on average. The nuclei measuring between 3–9 μm were around 609 in number and they constituted nearly 30% of the total cells. The medium-sized (10–15 μm nuclei ranged between 1176–1205 and constituted about 58.8% of the total nuclear content. The rest of the nuclei (large 20–22 μm) on average were found to be only about 229 in number and contributed relatively less (11.2%) to the total nuclear content of the ganglion. The large nuclei were confined to the periphery of the ganglion, while the small and medium-sized nuclei were distributed

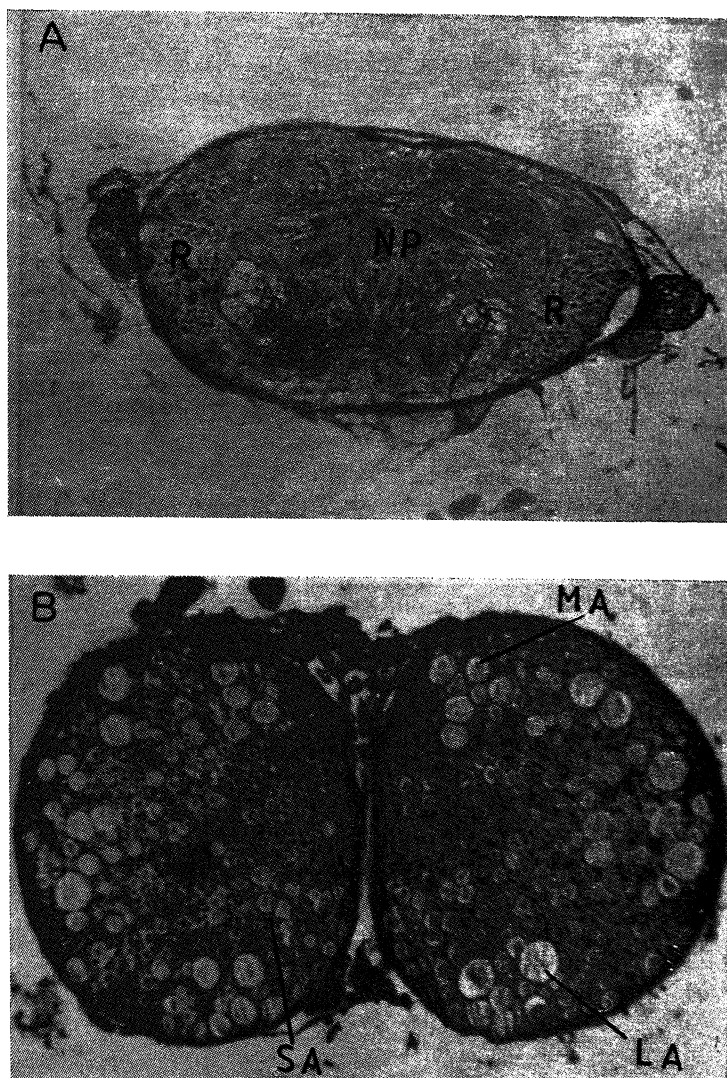


Figure 1. A. Transverse section of the seventh abdominal ganglion showing the peripheral rind (R) with different categories of cells, and the central fibrous core of neuropile (NP) $\times 40$. B. Transverse section of the connective between 6th and 7th abdominal ganglia, showing fibres of different diameters. Note the presence of large fibres (LA) along with medium (MA) and small (SA) fibres $\times 160$.

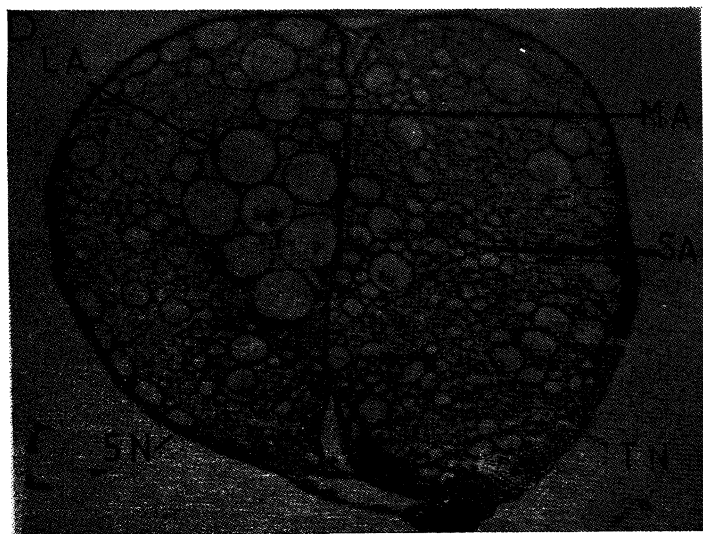
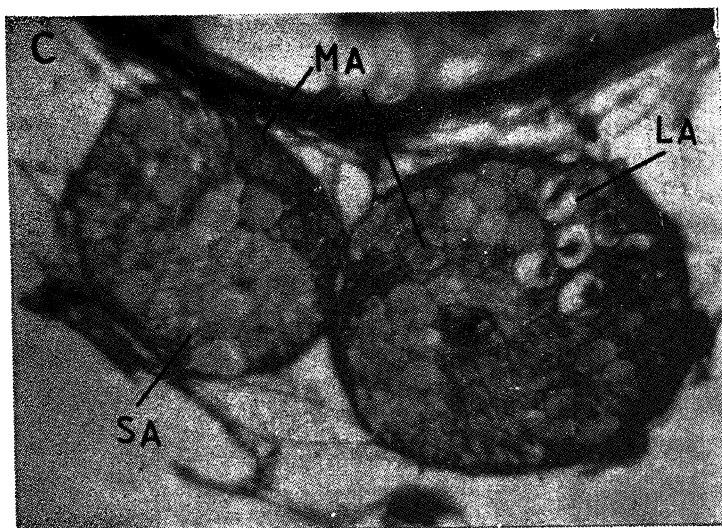


Figure 1. C. Transverse section of the 4th segmental nerve after its bifurcation, showing the two roots and their fibre content. Note the presence of large fibres (LA) along with medium (MA) and small (SA) fibres $\times 160$. D. A combined transverse section of 5th segmental (5N) and telsonic (TN) nerves, exhibiting fibres of different diameters. Note the presence of giant fibres (LA) along with medium (MA) and small (SA) fibres $\times 400$.

at random. The arrangement of the cells was so compact that the boundary of each cell could be resolved only under high magnification. By subtracting the number of motor and interneurons from the total cell count, the number of intra-ganglionic interneurons was obtained. Their number ranged from 1800–1830.

3.2. Microanatomy of the connective and peripheral nerves

Fibre counts and analyses were made for the connective and peripheral nerves such as the 4th and 5th segmental nerves and the telsonic nerve originating from the 7th abdominal ganglion. There was no significant bilateral difference between counterparts of either side. This similarity in composition was reinforced by the finding of consistent spatial positioning within the root of identifiable axons and certain medium and large fibres.

3.2a. *Connective*: The connective in its cross-section (figure 1B) exhibited a thick enveloping sheath (neural lamella) of $8\text{ }\mu\text{m}$ and its diameter ranged between $280\text{--}300\text{ }\mu\text{m}$. Table 1 shows the number of fibres that occurred in each of the four different diameter groups, and the fibre diameters used in counting were arranged in four descriptive groups, viz., fine fibres (less than $5\text{ }\mu\text{m}$), small fibres ($6\text{--}10\text{ }\mu\text{m}$), medium fibres ($11\text{--}15\text{ }\mu\text{m}$) and large fibres (greater than $16\text{ }\mu\text{m}$). Seven axons were above $16\text{ }\mu\text{m}$, with the largest axon having a diameter of $18\text{ }\mu\text{m}$ (table 1). These axons were not arranged into separate distinguishable bundles, but tended to occur dorsally, centrally, and a few distributed through the entire area of the connective. In contrast to this, the fine fibres along with the small fibres were found to be distributed uniformly in the connective whereas the medium sized fibres were sometimes arranged in pairs and in triplets and occupied various regions in the connective. The connective comprised of approximately 1460 fibres, falling into different sizes. The fine fibres were relatively larger in number (about 1259) and constituted 86% of the total fibre population. About 154 fibres were found to be in the diameter range of $6\text{--}10\text{ }\mu\text{m}$ and they contributed to

Table 1. Numerical distribution of fibres of different diameter ranges in the connective between the 6th and 7th abdominal ganglia of *H. fulvipes*.

Fibre diameter range in μm	Fibre population	Per cent in total fibre population
0-5	1259 ± 11.3	86
6-10	154 ± 11.1	10
11-15	41 ± 2.6	2.8
16-20	7 ± 1.5	0.5

Each value is an average of 4 counts of the same section, \pm standard deviation,

10% of the total population. The medium sized fibres were about 38 in number and they contributed to about 2.8%. The large fibres, about 7 in number, fall into the 4th category of 16–20 μm range, by far the lowest contribution to the total axon number.

3.2b. *4th segmental nerve*: Cross-section of the 4th segmental nerve (figure 1C) showed two separate and distinct roots, viz., the dorsal and ventral roots. The dorsal root measured 120–130 μm in diameter and was enclosed in a 6 μm thick neural lamella and the diameter of the ventral root ranged between 90–100 μm with a 5 μm thick covering sheath. The differences between the two roots were with regard to the size, the distribution and number of fibres in the nerve. The dorsal root comprised of about 10 large fibres and the ventral root consisted of about 7 large fibres. The two roots totally consisted of 290 fibres of which about 15 were large fibres and represented 5%. The rest of the fibre content consisted of about 20 medium-sized fibres constituting 7%, about 30 small fibres occupying 10% and the remainder (79%) represented by fine fibres (table 2).

3.2c. *5th segmental nerve*: The total diameter of the 5th segmental nerve close to its root measured 150 μm and was enclosed within a neural lamella of 5 μm diameter. This nerve in its cross-section (figure 1D) exhibited a total of approximately 620 fibres of different sizes. About 550 of these fibres were less than 5 μm and formed relatively a major contribution (87.4%) to the total axon number. Fibres of 6–10 μm contributed about 8%. Medium fibres ranging from 11–15 μm in diameter were about 10–12 in number and they represented 1.9%. The large fibres were about 8 in number and measured 16 μm and above in diameter (table 3). A majority of the large fibres were seen confined to the dorsal region and at the ventro-lateral region facing towards the telsonic nerve. The fine and small fibres were distributed uniformly throughout the nerve and the medium-sized fibres were observed to be located at various regions of the nerve and did not show specific pattern in their distribution.

Table 2. Numerical distribution of fibres of different diameter ranges in the 4th segmental nerve arising from the 7th abdominal ganglion of *H. fulvipes*.

Fibre diameter range in μm	Fibre population	Per cent in total fibre population
0–5	231 \pm 12.5	79
6–10	30 \pm 8.3	10
11–15	18 \pm 1.2	6
16–20	10 \pm 1.2	3.5

Each value is an average of 4 counts of the same section, \pm standard deviation.

Table 3. Numerical distribution of fibres of different diameter ranges in the 5th segmental nerve, arising from the 7th abdominal ganglion of *H. fulvipes*.

Fibre diameter range in μm	Fibre population	Per cent in total fibre population
0-5	540 ± 6.1	87.4
6-10	50 ± 4.8	8.0
11-15	12 ± 1.5	1.9
16-20	8 ± 0.1	1.3

Each value is an average of 4 counts of the same section, \pm standard deviation.

Table 4. Numerical distribution of fibres of different diameter ranges in the telsonic nerve, arising from the 7th abdominal ganglion of *H. fulvipes*.

Fibre diameter range in μm	Fibre population	Per cent in total fibre population
0-5	757 ± 10.7	87
6-10	61 ± 11.4	7
11-15	48 ± 3.1	5.5
16-20	20 ± 3.0	2.3

Each value is an average of 4 counts of the same section, \pm standard deviation.

3.2d. *Telsonic nerve*: The telsonic nerve (220 μm diameter) (figure 1D) like other peripheral nerves possessed an 8 μm thick enveloping sheath. It revealed a total fibre number of about 890, the majority of which belonged to the first category, the size ranging between 2-3 μm . These fine fibres represented 81% of the total fibre content. Fibres of 6-10 μm were about 50 in number and formed 7% of the total fibres. The fibres measuring 10-15 μm formed 5.5% of the total population of fibres. In contrast to other peripheral nerves the telsonic nerve comprised of more number of large (giant) fibres, approximately 20, and they measured more than 16 μm , and constituted about 2.3% (table 4). These large fibres occupied dorsal, mid-central and ventral regions of the nerve. The rest of the fibres were distributed at random.

4. Discussion

The general organization of the 7th abdominal ganglion as elucidated by the present study is in conformity with the pattern of organization of other invertebrate ganglia (Bullock and Horridge 1965), with a peripheral cellular rind and a central fibrous neuropile. The neuropile, regarded as the *terra incognita* of neuroanatomy (Bullock and Horridge 1965), is one of the most important regions of neural processing, integrating information from a variety of sources and effecting patterned outputs.

The sheathing around the ganglion and its nerves resembles that of other arthropods like cockroach (Pipa *et al* 1959; Wigglesworth 1959), locust (Cook 1951), etc., in being visible only under high magnification and also in the absence of a cellular perineurium beneath the neural lamella.

The occurrence of a relatively low number of large-sized cells and axons compared to the number of medium and small-sized neurons again goes well with the general plan of invertebrate neural organization. These relatively small number of 'giant' neurons are known to subserve the function of faster conduction of impulses (Bullock and Horridge 1965), and so very useful in quicker reflexes of the stinger in the present context. A role for the 7th ganglion in stinger reflexes in this scorpion was suggested earlier (Yellamma *et al* 1980). Further, the overall count of cells in the 7th ganglion in the present study has been found to be fairly larger than that reported from several other arthropods (Zawarzin 1924; Wiersma 1957; Backer 1962; Kendig 1967). The occurrence of larger number of neurons in any system naturally facilitates greater number of synaptic contacts and thus documents a high degree of integration (Bullock and Horridge 1965).

The fibre count of the connective between the 6th and 7th ganglia in the present study has been found to be fairly less compared to that in the cockroach (Nunne-macher *et al* 1974) and locust (Rowell and Dorey 1967). In keeping with the organization of the ganglion, the connective also showed only a few fibres larger than 15 μm and the majority of the fibres were less than 5 μm . This observation, however, coincides with that in the cockroach (Nunne-macher *et al* 1974) where majority of the fibres were less than 3 μm , and large fibres were relatively sparse. Fibres of less than 2 μm and 1 μm were reported in wax-moth pupa (Pipa and Woolver 1965) and in locust (Rowell and Dorey 1967) respectively. However, in the present study attempts to resolve fibres less than 1 μm by cobalt chloride back-filling were unsuccessful, since cobalt chloride could not diffuse into these axons so easily as to be feasible for compound microscopic studies. Basing on the work on crayfish connectives (Wiersma and Hughes 1961; Kennedy and Mellon 1964), it may be presumed here that many of these small fibres could be sensory, running through the connective.

Histological observations on peripheral nerves such as the 4th and 5th segmental and telsonic nerves also corroborated with those on the connective, in that majority of the fibres were less than 3 μm , and large fibres were relatively less.

These observations were further strengthened by cobalt chloride back-fillings showing only few fibres larger than 12 μm , with the remaining ranging between 3-10 μm in diameter. The total fibre count of these peripheral nerves, however, was far less compared to that of the 1st, 2nd and 3rd roots in the crayfish abdominal cord (Michael 1970).

Thus the present investigation on the 7th abdominal ganglion and its nerves in the scorpion *H. fulvipes* demonstrates that despite variations in the number of their different components and possibly accompanying subtle modifications in physiological functions, they conform in general to the organization met within the nervous systems of other invertebrates, especially arthropods.

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References

- Abercrombie M 1946 As cited by Mirrable A W 1962 The counting of cells and nuclei in microtome section ; *Q. J. Microsc. Sci.* **103** 331-347
- Babu K S 1965 Anatomy of the central nervous system of arachnids ; *Zool. Jb. Anat.* **82** 1-154
- Babu K S and Subhashini K 1981 Morphology of soma and dendrites of the giant fibre system in the sixth abdominal ganglion of the cockroach ; *J. Morphol.* **169** 351-355
- Backer H W 1962 The number of neurons in an insect ganglion ; *Experientia* **21** 719
- Bethe A 1897 Vergleichende Untersuchungen über die Funktionen des Zentralnervensystems der Arthropoden ; *Pflügers Arch. ges. Physiol.* **68** 449-545
- Bullock T H and Horridge G A 1965 *Structure and function in the nervous system of invertebrates* (San Francisco ; W H Freeman)
- Cook P M 1951 Observations on giant fibres of the nervous system of *Locusta migratoria* ; *Q. J. Microsc. Sci.* **92** 297-305
- Holmes W 1952 *A silver method for staining nerve axons, its application to sections and whole-mount preparations* ; Dept. of Zoology, University Museum, Oxford Neurophysiological Club
- Kendig J J 1967 Structure and function in the 3rd abdominal ganglion of the crayfish, *P. clarkii* ; *J. Exp. Zool.* **164** 1-20
- Kennedy D and Mellon D Jr 1964 Synaptic activation and receptive fields in crayfish interneurons ; *Comp. Biochem. Physiol.* **13** 275-300
- Michael C R 1970 Brain behaviour ; *Brain Behaviour Evol.* **3** 205-209
- Nunnemacher R F, Fiske W J and Sherman R G 1974 Size and number of nerve fibres in the central connectives of the cockroach *B. craniife* ; *J. Insect Physiol.* **20** 2123-2134
- Palmgren A 1948 A rapid method for selective silver staining of nerve fibres and nerve endings in mounted paraffin sections ; *Acta Zool.* **29** 377-392
- Pipa R L and Woolever P S 1965 Insect neurometamorphosis ; *Z. Zellforsch. Mick. Anat.* **68** 80-101
- Pipa R L, Cook L F and Richards A G 1959 Studies on the hexapod nervous system. II. The histology of the thoracic ganglia of the adult cockroach, *Periplaneta americana* (L.) ; *J. Comp. Neurol.* **113** 401-433
- Retzius G 1890 Zur Kenntniss des Nervensystems der Crustacean, in *Biologische Untersuchungen von Prof. Gustaf Retzius* ; NF. I 1-50 Central Druck, Stockholm
- Rowell C H F and Dorey A E 1967 The number and size of axons in the thoracic connectives of the desert locust, *S. gregaria* ; *Z. Zellforsch. Mick. Anat.* **83** 288-294
- Wiersma C A G 1957 On the number of nerve cells in a crustacean central nervous system ; *Acta Physiol. Pharm. Neerl.* **6** 135

- Wiersma C A G and Hughes G M 1961 On the functional anatomy of neural units in the abdominal cord of the crayfish, *P. clarkii* (Girard); *J. Comp. Neurol.* **116** 209-228
- Wigglesworth V B 1959 The histology of the nervous system of an insect, *Rhodnius prolixus* (Hemiptera). II. The central ganglia; *Q. J. Microsc. Sci.* **100** 100-299
- Yellamma K, Murali Mohan P and Babu K S 1980 Morphology and physiology of giant fibres in the seventh abdominal ganglion of the scorpion *Heterometrus fulvipes*; *Proc. Indian Acad. Sci. (Anim. Sci.)* **89** 29-38
- Zawarzin A 1924 Zur Morphologie der Nervenzentren des Bauchmarks der Insecten; ein Beitrag zur vergleichenden Histologie (Histologische Studien über Insecten VI); *Z. Wiss. Zool.* **122** 323-424

Branchial protein metabolism of freshwater fish *Tilapia mossambica* (Peters) during acute exposure and acclimation to sublethal alkaline water

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Abstract. Freshwater fish *Tilapia mossambica* (Peters) were exposed to sublethal alkaline water (pH 9.0) and the branchial protein metabolism was studied on acute exposure and acclimation. Branchial tissue had elevated structural and soluble protein fractions on acclimation which was correlated towards the gill hypertrophy. Proteolytic activity of the tissue was elevated on both acute exposure and acclimation. A/O ratio which forms a measure of ammonia formed per unit O_2 consumption was lesser on acclimation and hence mobilization of tissue free ammonia towards glutamine formation was suggested. The metabolic modulations have been correlated towards the positive survival value of the fish in alkaline waters.

Keywords. pH acclimation ; branchial metabolism ; structural proteins ; soluble proteins ; glutamine ; A/O ratio.

1. Introduction

Fish encounters abnormal pH levels of freshwater due to several factors like environmental pollution, addition of industrial effluents, hot springs, volcanic lakes, mine drainage and geological pattern of natural changes (McKee and Wolf 1963 ; Beamish 1972 ; Cairns *et al* 1972 ; Dovland *et al* 1976 ; Oden 1976 ; Dillon *et al* 1978). These pH changes affect wild fish populations in many freshwater lakes, streams and rivers (Anderson *et al* 1971 ; Beamish and Harvey 1972 ; Jensen and Snekvik 1972 ; Almer *et al* 1974 ; Schofield 1975 ; Karuppasamy 1979).

Studies on fish in altered pH media have been undertaken regarding tolerance levels (Bandt 1936 ; Trama 1954 ; Carter 1964 ; Jordan and Lloyd 1964 ; Beamish 1972 ; Daye and Garside 1975), O_2 consumption (Packer and Dunson 1972 ; Krishna Murthy *et al* 1980) survival and development of embryos and histopathological changes of tissues (Daye and Garside 1976, 1980a, b) and physiological aspects (Packer and Dunson 1970, 1972 ; Lievestad and Muniz 1976).

However there has been little information on tissue metabolism of fish exposed and acclimated to alkaline media. Our previous studies revealed that the pHs 10.5 and 3.5 were lethal limits to the fish *Tilapia mossambica* (Krishna Murthy *et al* 1980) and tissue metabolism was drastically altered (Bhaskara Haranath *et al* 1978).

The animals develop compensatory changes in tissue metabolism under stress conditions (Precht 1958 ; Kanungo and Prosser 1959 ; Das and Prosser 1967 ; Govindappa and Rajabai 1976). Hence it was felt desirable to understand the possible tissue metabolic modulations during acute exposure and acclimation to sublethal alkaline waters. Since the branchial tissue participates in immediate ionic regulations, it will be worthwhile to study the metabolic changes of this tissue under induced alkaline stress.

2. Materials and methods

Freshwater fish, *T. mossambica* (Peters) of 10 ± 1 g weight, were acclimatized in glass aquaria with flowing dechlorinated water to the laboratory conditions (25°C , pH 7.0 ± 0.2 ; and light period of 12 hr). The fish were fed with formulated diet of commercial fish pellets. The test fish were exposed to extreme sublethal alkaline pH medium (pH 9) which was prepared by adding 10^{-1} N NaOH as suggested by Krishna Murthy *et al* (1980) and the pH was checked with pH meter (Elico model LI-10 Hyd.).

The fish were divided into three groups. viz., controls, acute exposed and acclimated. The control fish was maintained in normal tap water at pH 7 ± 0.2 and the second and third groups of fish were exposed to pH 9 ± 0.1 for one day (acute exposed) and for 15 days (acclimated) (Krishna Murthy *et al* 1980). The fish were sacrificed separately, the gill was isolated, rapidly chilled and employed for biochemical analysis.

Total, soluble and structural proteins were estimated by the method of Lowry *et al* (1951) and protease activity (neutral) and free amino acid levels by the method of Moore and Stein (1954). The rate of tissue respiration was measured using the conventional Warburg constant volume respirometric apparatus (Umbreit *et al* 1959). Glutamate dehydrogenase (E.C. 1.4.1.3) activity was estimated by the method of Lee and Lardy (1965) as modified by Reddanna and Govindappa (1979). Free ammonia, urea and glutamine levels were estimated by the methods described by Bergmeyer (1965), Natelson (1971) and Colowick and Kaplan (1957) respectively. A/O ratios were calculated by dividing free ammonia contents with tissue O_2 consumption.

3. Results

Data are presented in tables 1 and 2. On acute exposure to sublethal alkaline medium the branchial tissue had depleted total protein (TP) content (table 1). The soluble protein (SP) fraction was considerably elevated while structural protein (StP) was depleted. Protease activity was highly elevated with significant increase in free amino acid content. The ratios of soluble proteins to structural

Table 1. Levels of total, soluble and structural proteins, free amino acid content, protease activity and ratios of SP/TP, StP/TP, SP/StP in the branchial tissue of control, acute exposed and acclimated fish.

Parameter (mg/g/ wet wt)	Control		Acute exposed		Acclimated
Total proteins (TP)	94.60 ±8.21		83.97 ±6.4		111.55 ±8.95
		-11.24 <i>P</i> < 0.001		-17.92 <i>P</i> < 0.001	
Soluble proteins (SP)	32.15 ±2.85		43.10 ±3.84		42.12 ±4.18
		+34.06 <i>P</i> < 0.001		+31.01 <i>P</i> < 0.001	
Structural proteins (StP)	62.45 ±4.86		40.87 ±4.21		69.43 ±5.62
		-31.56 <i>P</i> < 0.001		+11.18 <i>P</i> < 0.01	
Protease μ mol tyrosine/mg protein/hr	0.0064 ±0.00071		0.039 ±0.0028		0.024 ±0.0016
		+509.4 <i>P</i> < 0.001		+275 <i>P</i> < 0.001	
Free amino acids	17.91 ±1.84		25.2 ±2.32		34.65 ±3.85
		+40.93 <i>P</i> < 0.001		+92.91 <i>P</i> < 0.001	
SP/TP	0.340		0.51		0.38
		+50.20		+18.81	
StP/TP	0.66		0.49		0.62
		-25.86		- 6.09	
SP/StP	0.515		1.055		0.606
		+105.28		+17.74	

The values are mean of 6 observations; Mean \pm S.D.; + and - indicate per cent increase and decrease respectively on the control values. *P* denotes statistical significance.

proteins (SP/StP) and soluble proteins to total proteins (SP/TP) were higher and StP/TP was lesser than controls. The tissue oxygen consumption was considerably high (table 2). A/O ratio was slightly lesser and GDH activity was highly inhibited. Free ammonia and urea contents had non-significant changes while glutamine content was depleted. However, on acclimation, the levels of total, soluble and structural proteins were significantly elevated. Tissue protease was activated and free amino acid content was elevated. The SP/TP and SP/StP

Table 2. Levels of O₂ consumption, ammonia/O₂ ratio, glutamate dehydrogenase, free ammonia, urea and glutamine and ratios of urea/ammonia, glutamine/ammonia in the branchial tissue of control, acute exposed and acclimated fish.

Parameter	Control	Acute exposed	Acclimated
Tissue oxygen consumption μl of O ₂ /g.wt./hr	309.15 ±25.12	397.96 ±29.85	324.05 ±26.52
		+28.73 P < 0.001	+ 4.82 NS
A/O ratio	0.0082	0.0077	0.0055
		- 6.1	- 32.95
GDH μ mole of formazan/ mg protein/hr	0.029 ±0.001	0.012 ±0.001	0.033 ±0.002
		- 58.62 P < 0.001	+13.79 P < 0.001
Free ammonia μ moles/g. wt.	2.55 ±0.35	2.65 ±0.28	3.11 ±0.34
		+ 3.94 NS	+21.96 P < 0.01
Urea μ moles/g. wt.	4.15 ±0.18	4.11 ±0.25	2.81 ±0.14
		- 1.44 NS	- 25.86 P < 0.001
Glutamine μ moles/g. wt.	113.83 ±10.25	96.57 ±2.19	134.24 ±13.12
		- 8.83 P < 0.001	+26.74 P < 0.01
Urea/Ammonia	1.486	1.612	0.904
		+ 8.51	- 39.33
Glutamine/Ammonia	44.64	37.87	43.16
		- 15.23	- 3.33

Each value is mean of 6 observations; Mean ± S.D.; + and - indicate per cent increase and decrease respectively from controls. *P* denotes statistical significance and 'NS' is non-significant.

ratios were considerably higher while that of StP/TP were lower than controls. Tissue oxygen consumption had non-significant change with low A/O ratio as compared to control. GDH activity level was elevated. While the free ammonia and glutamine levels were significantly increased, urea content was depleted. The urea/ammonia ratio was considerably lesser than the control value.

4. Discussion

The branchial protein metabolism showed differential pattern during acute exposure and acclimation to sublethal alkaline waters.

Acute exposure of fish to sublethal alkaline waters depleted total protein content of the gill. In view of highly elevated protease activity, depleted protein content in the tissue can be envisaged. However, soluble protein fraction was elevated while the structural protein fraction was depleted, suggesting possible alterations in the solubility properties of the proteins in the tissue. Higher SP/TP and SP/StP ratios indicate that the soluble protein fraction was elevated, probably a prerequisite for proteolysis in the tissue. Consequently, the structural proteins of the tissue depleted suggesting proteolysis at structural level of organization of gill. These observations agree with earlier reports where high tissue proteolysis was recorded in liver and muscles of fish exposed to alkaline waters (Bhaskara Haranath *et al* 1978) and histopathological changes in tissues of fish exposed to extreme pH of the medium (Daye and Garside 1976). Since protease activity was high, the tissue free amino acid content increased considerably. The oxygen consumption was elevated with lesser A/O ratio suggesting possible suppression of oxidations of protein components in the tissue. In view of the highly inhibited GDH activity, which forms an index of amino acid oxidations, the decreased mobilization of amino acids into oxidations can be envisaged. Consequently, free ammonia and urea levels had non-significant change from the controls.

However, on acclimation the branchial total protein content was significantly elevated suggesting the onset of either enhanced protein biosynthetic mechanisms, or decreased proteolysis in the tissue. Since protease activity was also elevated, increased protein content might be due to stepped-up protein biosynthesis, with active turnover of tissue proteins. In the light of widely reported mucification and hypertrophy of the branchial tissue in altered pH media (Daye and Garside 1980b) active protein synthesis can be visualised. Since both soluble and structural protein fractions were elevated, accumulation of proteins at structural and dynamic levels of organization of gill can be expected. Free amino acid content was elevated, which may be due to increased proteolysis.

Tissue oxygen consumption had non-significant change, while A/O ratio was far lower than the control, suggesting lesser mobilization of protein components into oxidations or mobilization of ammonia into other components. However, GDH activity was considerably elevated indicating the involvement of amino acids in oxidative reactions. Free ammonia content was increased due to high oxidative deamination reactions in the tissue. Glutamine content was considerably high with decrease in urea, suggesting the mobilization of tissue ammonia towards the formation of glutamine, which may be responsible for the lower A/O ratio.

In general it can be concluded that the branchial tissue, on acclimation in sublethal alkaline waters, accumulates proteins leading to hypertrophy of the tissue which might provide positive survival value for the fish in imposed alkaline stress.

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References

- Almer B, Dickson W, Ekstrom C, Hornstrom E and Miller U 1974 Effects of acidification of Swedish lakes; *Ambio* 3 30-36
- Anderson G, Gustafson K J and Lindstrom T 1971 Rodingen I Rosjoarna Pa Fulufjall. Drottningholm: *Freshwater Research Laboratory Sweden Information* 8 9
- Bandt H J 1936 Der Fur Fische "todliche pH Wert" in alkalischem Bereich; *Z. Fisch. Deren Hilfswiss* 34 359-361
- Beamish R J 1972 Lethal pH for the white sucker *Catostomus commersoni* (Lacepede); *Trans. Am. Fish Soc.* 101 355-358
- Beamish R J and Harvey H H 1972 Acidification of the Lacloche mountain lakes, Ontario and resulting fish mortalities; *J. Fish. Res. Board Can.* 29 1131-1143
- Bergmeyer H U 1965 *Methods of biochemical analysis* (New York and London: Academic Press)
- Bhaskara Haranath V, Reddanna P and Govindappa S 1978 Effects of exposure to altered pH media on tissue proteolysis and nitrogenous products in a freshwater fish *Tilapia mossambica* (Peters); *Indian J. Exp. Biol.* 16 1088-1090
- Cairns J, Dickson K L and Crossman J S 1972 The response of aquatic communities to spills of hazardous materials; *Proc. 1972 Natl. Conf. Hazardous materials spills* pp. 179-197
- Carter L 1964 Effects of acidic and alkaline effluents on fish in sea water; *Effluent water Treat. J.* 4 484-486
- Colowick S P and Kaplan N O 1957 *Methods in enzymology* (New York: Academic Press) 3 501
- Das A B and Prosser C L 1967 Biochemical changes in tissues of gold fish acclimated to high and low temperatures. I. Protein synthesis; *Comp. Biochem. Physiol.* 21 449-467
- Daye P G and Garside E T 1975 Lethal levels of pH for brook trout, *Salvelinus fontinalis* (Mitchill); *Can. J. Zool.* 53 639-641
- Daye P G and Garside E T 1976 Histopathological changes in surfacial tissues of brook trout, *Salvelinus fontinalis* (Mitchill) exposed to acute and chronic levels of pH; *Can. J. Zool.* 54 2140-2155
- Daye P G and Garside E T 1980a Structural alterations in embryos and alevins of the Atlantic salmon, *Salmo salar* L. induced by continuous or short-term exposure to acidic levels of pH; *Can. J. Zool.* 58 27-43
- Daye P G and Garside E T 1980b Development, survival and structural alterations of embryos and alevins of Atlantic salmon, *Salmo salar* L. continuously exposed to alkaline levels of pH from fertilization; *Can. J. Zool.* 58 369-377
- Dillon P J, Jeffries D S, Snyder W, Reid R, Yan N D, Evans D, Moss J and Scheider W A 1978 Acidic precipitation in South-Central Ontario: recent observations; *J. Fish. Res. Board Can.* 35 809-815
- Dovland H, Joranger E and Semb A 1976 Deposition of air pollutants in Norway; in *Impact of acidic precipitation on forest and freshwater ecosystems in Norway*; (ed) F H Brekke SNSF Project FR 6/76 14-35
- Govindappa S and Rajabai B S 1976 Some aspects of protein metabolism in crab *Paratelphusa hydrodromus* (Herbt) during cold acclimation; *J. Anim. Morphol. Physiol.* 23 76-84
- Jensen K W and Snekvik E 1972 Low pH levels wipe out salmon and trout population in southernmost Norway; *Ambio* 1 223-225
- Jordan D H M and Lloyd R 1964 The resistance of rainbow trout [*Salmo gairdneri* (Richardson)] and roach (*Rutilus rutilus* L.) to alkaline solutions; *Int. J. Air Water Pollut.* 8 405-409
- Kanungo M S and Prosser C L 1959 Physiological and biochemical adaptation of gold fish to cold and warm temperatures. I. Standard and active oxygen consumptions of cold and warm acclimated gold fish at various temperatures; *J. Cell Comp. Physiol.* 54 259-263
- Karuppasamy P 1979 Pollution and fish mortality in Chaliyar river, Mavoor (near Calicut) from 7-3-1979 to 16-3-1979; *Marine Fisheries Information Service* 7 11-13

- Krishna Murthy V, Reddanna P and Govindappa S 1980 Hepatic carbohydrate metabolism in *Tilapia mossambica* (Peters) acclimated to low environmental pH; *Can. J. Zool.* **59** 400-404
- Lee Y L and Lardy H A 1965 Influence of thyroid hormones on L-glycerophosphate dehydrogenases and other dehydrogenases in various organs of the rat; *J. Biol. Chem.* **240** 1427-1432
- Liestad H and Muniz I P 1976 Fish Kill at low pH in a Norwegian river; *Nature (London)* **259** 391-392
- Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with Folin phenol reagent; *J. Biol. Chem.* **193** 265-275
- McKee J E and Wolf H W 1963 *Water quality criteria* (Sacramento, Calif.: Water Quality Control Board Publ. 3-A)
- Moore S and Stein W H 1954 A modified ninhydrin reagent for the photometric determination of aminoacids and related compounds; *J. Biol. Chem.* **211** 907-913
- Natelson S 1971 *Techniques in clinical chemistry* (Illinois: Charles C. Thomas, Publishers) pp. 146
- Oden S 1976 The acidity problem—an outline of concepts; *Proc. 1st Int. Symp. on acid Precip. Forest Ecosystems*; in special issue; *J. Water Air Soil Pollut.* pp. 1-36
- Packer R K and Dunson W A 1970 Effects of low environmental pH on blood pH and Na⁺ balance of brook trout; *J. Exp. Zool.* **174** 65-72
- Packer R K and Dunson W A 1972 Anoxia and sodium loss associated with death of brook trout; *Comp. Biochem. Physiol.* **A41** 17-26
- Precht H J 1958 Concept of temperature adaptation of unchanging reaction systems of cold blooded animals; In *Physiological adaptation* (ed.) C L Prosser (Washington D.C.: American Physiological Society)
- Reddanna P and Govindappa S 1979 Effects of *in vivo* muscular stimulations. II. Influence on hepatic carbohydrate metabolism; *J. Anim. Morphol. Physiol.* **26** 156-161
- Schofield C L 1975 Lake acidification in the Adirondack Mountains of New York: Causes and consequences; *Proc. 1st Int. Symp. Acid Precip. Forest Ecosystems*; Ohio State University
- Trama F B 1954 The pH tolerance of the common bluegill (*Lepomis macrochirus* Rafinesque); *Not. Nat. Acad. Nat. Sci. Philadelphia* **256** 1-13
- Umbreit W W, Burris R H and Stauffer J F 1959 *Manometric techniques* (Minneapolis: Burgess Publishing)

Temperature-related chromosome polymorphism in *Drosophila ananassae*

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Abstract. Correlated studies on the influence of temperature in the frequency of inversions in the *D. ananassae* population of Golabandha shows that temperature fluctuation has a positive bearing on 2LA inversion while negatively so with respect to 3LA and 3RA inversions.

Keywords. *Drosophila ananassae*; inversion; 2LA; 3LA; 3RA.

1. Introduction

Clear evidences exist to sustain the evolution of differential gene arrangements in species of *Drosophila* to meet the adaptive needs in a dynamic environment. In as much as the adaptive values of different genomes differ considerably, the fitness of certain kinds of gene arrangements may, therefore, decrease or increase with fluctuation in environmental milieu. *D. ananassae*, a cosmopolitan domestic species, is known to be invested with a large number of inversions in its natural population (Kaufmann 1936; Kikkawa 1938; Dobzhansky and Dreyfus 1943; Shirai and Moriwaki 1952; Seecof 1957; Freire-Maia 1961; Ray-Chaudhuri and Jha 1966; Singh and Ray-Chaudhuri 1969; Sreeram Reddy and Krishnamurthy 1969, 1970; Sajjan and Krishnamurthy 1970; Singh 1970; Siddaveere Gowda and Krishnamurthy 1971). Again, of the several paracentric inversions, 2LA, 3LA and 3RA (Rajeswari and Krishnamurthy 1969), or their equivalent alpha, delta and eta (Ray-Chaudhuri and Jha 1966) are common to all populations of this species (Singh 1970), while all other inversions are selectively restricted to these populations. Certain populations of *Drosophila* undergo seasonal changes with respect to their chromosomal composition which, however, varies in intensity (Carson and Stalker 1949; Spiess 1950). For instance, Levitan (1951, 1957) reported marked seasonal fluctuation in the frequency of inversions in *D. robusta* population of Virginia while Carson's (1958) data on the same species endemic to Missouri are quite contradictory in being insignificant. Epling *et al* (1953) have argued that seasonal changes of gene arrangement in the chromosomes promote the adaptive values of the inversions which in turn influence the

nature and frequency of the polymorphism itself. In an attempt to assess the correlation if any, between the frequency of different inversions and the environmental temperature, the present study has been undertaken.

2. Materials and methods

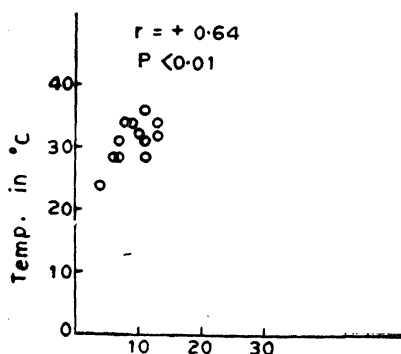
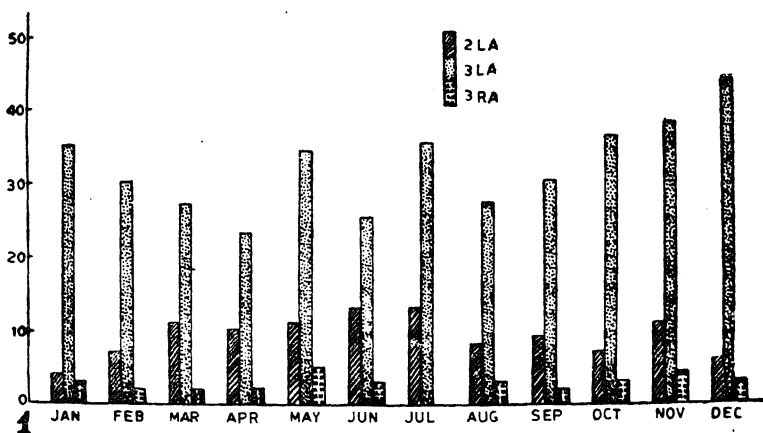
The flies were collected from the natural population of *D. ananassae* of Gola-bandha situated at an altitude of 17.5 m and about 6 km to the south of the University campus. Collections were made in the first week of every month on fermented banana bait in glass bottles. Fertilised females collected from nature were transferred to independent vials with wheat cream agar media. Chromosomal polymorphism was studied from the salivary glands of a hundred larvae from lacto-aceto-orcein squash preparations.

3. Results

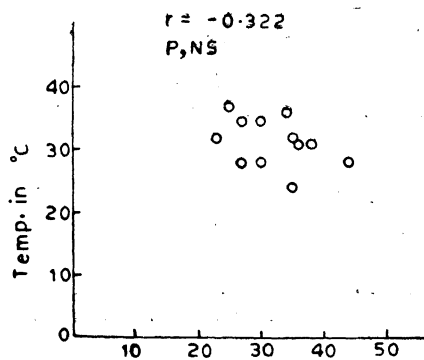
Table 1 represents the percentage of homo- and hetero-karyotypes of *D. ananassae* in different months, i.e., January to December. The percentage of homokaryotypes were more than 50 every month while that of the heterokaryotypes ranged between 31 and 48. The frequency of the three commonly occurring inversions, as found in this population, are represented in figure 1. It is observed that the frequencies of 2LA inversion vary between 4% (in January) and 13% (in June and July) and that of 3RA between 2% (in February, March, April and September) and 5% (in May) while the percentage of 3LA inversion varies between 23% (in April) and 44% (in December) in a year. Moreover, it has been marked that 3RA inversion is completely absent in the population in the month of July.

4. Discussion

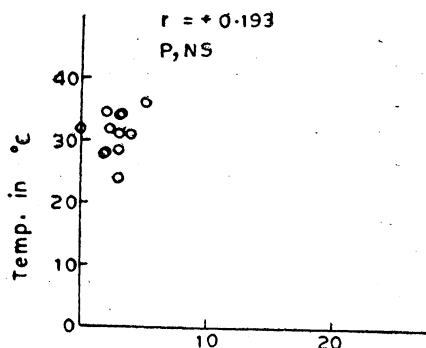
Extensive qualitative chromosomal variability has no doubt been reported in *D. ananassae* but unfortunately the information on the frequencies of these qualitative chromosomal variabilities and their correlation, if any, with the fluctuation of environmental factors is extremely meagre (Dobzhansky 1947; Stalker and Carson 1948; Carson and Stalker 1949; Spiess 1950; Battaglia and Birch 1956; Carson 1967). Curiously however the data of Dobzhansky (1956) on *D. pseudoobscura* while indicating seasonal fluctuations in the frequency of chromosomal composition, those of Battaglia and Birch (1956) on *D. willistoni* deny such correlation. In our studies, what is still more intriguing, the annual temperature fluctuation has positive bearing on 2LA inversion but negatively so with respect to 3LA and 3RA inversions (figures 2, 3 and 4). Indeed if this is proved to be a widely occurring phenomenon, then we must conclude that the inversions in their very nature confer such 'position effects' as seemingly contribute to the homeostatic mechanism of the species.



2 Frequency of 2LA inversion



3 Frequency of 3LA inversion



4 Frequency of 3RA inversion

Figures 1-4. 1. Histogram showing the frequency of different types of inversions in *D. ananassae*. 2-4. Correlation between inversion and environmental temperature. 2. 2LA inversion. 3. 3LA inversion. 4. 3RA inversion.

Table 1. Number of heterokaryotypes of *Drosophila ananassae* in different months.

Months	Average temp. in °C	% Heterokaryo- types*
January	24	42
February	28	31
March	28	36
April	32	34
May	36	43
June	34	35
July	32	48
August	34	37
September	34	38
October	31	42
November	31	45
December	28	47

* 100 larvae were examined every month.

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References

- Battaglia B and Birch L C 1956 *Nature (London)* **178** 1005 (from *Science* 1966) **152** (3723) 711
- Carson H L 1958 The population genetics of *Drosophila robusta*; *Adv. Genet.* **9** 1-40
- Carson H L 1967 Chromosomal polymorphism in altitudinal races of *Drosophila*; *Proc. Jpn. Soc. Syst. Zool.* **3** 10-16
- Carson H L and Stalker H D 1949 Seasonal variation in gene arrangement frequencies over a three year period in *Drosophila robusta* Sturtevant; *Evolution* **3** 322-329
- Dobzhansky Th 1947 Genetic structure of natural populations; *Yearb. Carnegie Inst.* **46** 155-165
- Dobzhansky Th 1956 Genetics of natural populations. XXV. Genetic changes in populations of *Drosophila pseudoobscura* and *Drosophila persimilis* in some localities in California; *Evolution* **10** 82-92
- Dobzhansky Th and Dreyfus A 1943 Chromosomal aberrations in Brazilian *Drosophila ananassae*; *Proc. Natl. Acad. Sci. U.S.A.* **29** 301-305
- Epling C, Mitchell D F and Martoni R H T 1953 On the role of inversions in wild populations of *Drosophila pseudoobscura*; *Evolution* **7** 342-365
- Freire-Maia N 1961 Peculiar gene arrangements in Brazilian natural populations of *Drosophila ananassae*; *Evolution* **15** 486-495
- Kaufmann B P 1936 A terminal inversion in *Drosophila ananassae*; *Proc. Natl. Acad. Sci. USA* **22** 591-594
- Kikkawa H 1938 Studies on the genetics and cytology of *Drosophila ananassae*; *Genetica* **20** 458-516

- L vitan M 1951 Selective differences between males and females in *Drosophila robusta*; *Am. Nat.* 85 385-388
- Levitan M 1957 Natural selection for linked gene arrangements; *Anat. Rec.* 127 430
- Rajeswari P and Krishnamurthy N B 1969 Inversion polymorphism in different populations of *Drosophila ananassae* from Mysore State; *Indian J. Heredity* 1 143-147
- Ray-Chaudhuri S P and Jha A P 1966 Studies on the salivary gland chromosomes of Indian *Drosophila ananassae*; *Proc. Int. Cell Biol. Meet. Bombay* pp. 352-383
- Sajjan S N and Krishnamurthy N B 1970 Report on two new translocations in a natural population of *Drosophila ananassae* from Heriyar (Mysore State, India); *DIS* 45 166
- Seecof R L 1957 Cytological analysis, section III from XXII genetic studies in irradiated natural populations of *Drosophila*; Univ. Texas Publications No. 5721 pp. 261-281
- Shirai M and Moriwaki D 1952 Variations of gene sequence in various strains of *Drosophila ananassae*; *DIS* 26 120-121
- Siddaveere Gowda L and Krishnamurthy N B 1971 Inverted gene arrangements in *Drosophila ananassae* population of Western Ghats; *Science J. Mysore Univ.* 24 115-119
- Singh B N 1970 Distribution of most common inversions of *Drosophila ananassae* in different parts of India including Andaman and Nicobar Islands; *Indian Biol.* 2 78-81
- Singh B N and Ray-Chaudhuri S P 1969 Geographic differentiation in natural populations of *Drosophila ananassae*; *Abstr. III Cell Biol. Conf. Delhi* pp. 32
- Spieß E 1950 Experimental populations of *Drosophila persimilis* from an altitudinal transect of the Sierra Nevada; *Evolution* 4 14-33
- Sreeram Reddy G and Krishnamurthy N B 1969 Inversion heterozygosity in two populations of *Drosophila ananassae*; *Proc. 56th Indian Sci. Cong. Assn.* pp. 451
- Sreeram Reddy G and Krishnamurthy N B 1970 Studies with variants of the gene arrangements in Goan and Karwar populations of *Drosophila ananassae*; *Proc. 57th Indian Sci. Cong. Assn.* pp. 362
- Stalker H D and Carson H L 1948 An altitudinal transect of *Drosophila robusta* Sturtevant; *Evolution* 2 295-305

Life and fecundity tables for the longicorn beetle borer, *Olenecamptus bilobus* (Fabricius) (Coleoptera : Cerambycidae)

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Abstract. The present paper deals with the life and fecundity tables for the cerambycid borer, *Olenecamptus bilobus* (Fabricius). Under a given set of conditions and food supply, the population of this species increased with an infinitesimal and a finite rate. The population increased by 20.41 times between two successive generations and 86.42 days were taken to complete one generation. The adults constituted only 1.29% to the population of the stable age, while eggs, larvae, pupae and dormant adults contributed to the extent of 24.95, 68.22, 4.38 and 1.16% respectively.

Keywords. Life and fecundity tables; *Olenecamptus bilobus* (Fabricius); *Artocarpus chaplasha* Roxb.; stable age-distribution.

1. Introduction

The longicorn beetle borer, *Olenecamptus bilobus* (Fabricius), is found predominantly in the Oriental region with an extended distribution up to the Papuan and Malagasy subregions. The species is one of the most common sap-wood borers of a number of dead or dying timber yielding plants. The bionomics and life-history of this species have been dealt with by Khan and Maiti (1980). The observations reported here, are concerned with the life and fecundity tables, including the stable age-distribution of the species. The infinitesimal (r_m) and finite (λ) rates of increase, net reproductive rate (R_0) and the mean generation time were the basic parameters used in the present communication to assess the population growth in the laboratory.

2. Material and methods

The present study was based on the material collected during the period of 1978-80 under a research project on the "Ecological interaction and economic status of the xylophagous insects of the Islands of Andaman and Nicobar", under the guidance of one of us (P K Maiti). During the course of the study, a number of logs of *Artocarpus chaplasha* Roxb., infested with the immature stages of *O. bilobus*,

was collected from several field sites of South Andaman and held in galvanized iron cages (70 cm \times 37 cm \times 37 cm) for the emergence of the adult beetles in the laboratory. The newly emerged beetles were sexed and 50 pairs of males and females were each separately kept in glass breeding-cages (36 cm \times 22 cm \times 22 cm), containing a layer of moist sandy soil at the bottom. Moist sandy soil was however, kept to minimise the loss of moisture from the breeding-cages. The beetles were provided with fresh green leaves and twigs of *Ficus religiosa* L. for food and freshly cut billets, measuring 25-30 cm in length and 8-12 cm in diameter, of *Artocarpus chaplasha* Roxb. for oviposition, both of which were renewed everyday between 900 and 1000 hr IST. The number of oviposition slits on the billets was counted and the total number of eggs laid thereon was recorded.

Each day, the infested billets from the breeding-cages were assigned a batch-number and placed in a galvanized iron cage (similar to those used for rearing the immature stages collected from the field sites), containing a layer of moist sandy soil at the bottom. In this case, however, the moist sandy soil was used to prevent over desiccation from the infested billets, so that they could retain the moisture content for a longer period for proper development of the progeny. Beginning from the third day following oviposition up to the completion of development of the progeny, three sample billets were taken out and dissected every alternate day between 1200 and 1400 hr IST, to study the development of egg, larva and pupa and other relevant phenomena. The adults, which emerged on a particular day, were transferred to separate cages for oviposition to determine the age-specific fecundity. The average fecundity of the females on subsequent days was recorded until all the females died. Since, the sex-ratio was 2♀:1♂ (based on 1600 adults), the number of eggs laid per female was multiplied by 2/3 to get the number of female births (m_x). Life and fecundity tables were constructed according to Birch (1948), elaborated by Howe (1953), Laughlin (1965) and Atwal and Bains (1974). The innate capacity of increase (r_m) and finite rate (λ) were calculated. The values of x (pivotal age in days), L_x (survival of females at different age intervals), and m_x (number of female progeny per female) were worked out. Observations were also made on the stable age-distribution (per cent distribution) of various age groups by calculating the birth-rate and death-rate when reared in a limited space.

During the course of these studies in the laboratory, the maximum and minimum temperatures recorded were 29.7°C and 26.5°C respectively, while the relative humidity prevailed between 67.5% and 93.0%.

3. Results and discussion

The maximum duration of incubation, development of larva, pupa and dormant adult has been observed to be 5, 50, 15 and 7 days respectively. Present observations on the duration of different developmental stages correspond strikingly with those observed by Khan and Maiti (1980). The number of individuals survived between different developmental stages is presented in table 1. From the data presented in the table the developmental survival rate has been estimated at 0.441.

The figures of the mature females emerged from the immature stages have been pooled and a grouping of a day interval of age is employed. The results are

Table 1. Survival of different developmental stages of *Olenecamptus bilobus* (Fabr.) on *Artocarpus chaplasha* Roxb.

Batch No.	No. of eggs	Number survived			
		Egg period (0-5 days)	Larval period (6-55 days)	Pupal period (56-70 days)	Dormant adult period (71-77 days)
1	379	334	235	197	173
2	456	390	274	230	205
3	503	434	303	263	233
4	412	348	244	201	175
5	517	429	290	242	204
6	450	396	280	233	206
7	391	341	241	200	178
8	342	286	203	169	149
9	401	341	238	199	176
10	279	237	171	143	131
Total	4148	3536	2479	2077	1830

Table 2. Observed, as well as, smoothed distribution of mortality among the mature females of *Olenecamptus bilobus* (Fabr.).

Age of the mature females in days x	Observed l_x	Smoothed l_x	Observed d_x	Smoothed d_x
1	854	854.00	24	45.68
2	830	808.32	60	70.63
3	770	737.69	100	88.70
4	670	648.99	95	98.55
5	575	550.44	94	100.44
6	481	450.00	101	95.21
7	380	354.79	89	85.17
8	291	269.62	78	72.05
9	213	197.57	60	58.25
10	153	139.32	50	44.32
11	103	95.00	36	32.63
12	67	62.37	20	22.90
13	47	39.47	17	15.39
14	30	24.08	16	9.92
15	14	14.46	14	14.46
			854	854.00

$$X^2 = 15.86; n = 12; P = (0.20), 0.10; l_x = 869.77e^{-0.0183001x^2};$$

presented in table 2, where the customary symbols have been used, i.e., l_x being the number of individuals which survive to the age x and d_x the number dying between the ages x and $x + 1$, so that in the present table,

$$d_x = l_x - l_{x+1}.$$

The raw data have then been smoothed. For smoothening of the raw data, there are many a satisfactory method of which the following one seems to be most convenient :

Defining μ_x as the force of mortality at the age x ;

$$-\frac{1}{l} \frac{dl_x}{dX} = \mu_x.$$

Now, if the force of mortality is assumed to be directly proportional to the age and $\mu_x = 2h^2 X$, where $2h^2$ is a constant, then by integration we get

$$l_x = l_0 e^{-h^2 x^2}. \quad (1)$$

Putting $l_x/l_0 = Q_x$, and $Q_x = 1 - P_x$,

$$Q_x = 1 - e^{-h^2 x^2},$$

and, by differentiation we have,

$$dQ_x = 2h^2 X e^{-h^2 x^2} dX ;$$

the probability of dying between the ages X and $X + dX$ being given by the right-hand member of the last equation. Then if M be the mean age at death,

$$M = 2h^2 \int_0^{\infty} X^2 e^{-h^2 x^2} dX,$$

from which, by integration we get,

$$M = \frac{\sqrt{\pi}}{2h}.$$

The observed and the smoothed values of l_x have been presented in table 2, and in testing the agreement between the smoothed and observed l_x , Chi-Square test is employed. It has been observed that $X^2 = 15.86$ and for $n = 12$, $P = (0.20)$, 0.10 , which shows that the fit is satisfactory. As a matter of interest, it might be worth mentioning that the same type of equation has been found to give an excellent fit in the case of 524 female vestigial *Drosophila* (data from Pearl and Parker 1924) and in case of 119 voles (*Microtus agrestis*) (Leslie and Ranson 1940).

The life and fecundity tables for *O. bilobus* on *Artocarpus chaplasha* Roxb. based on the smoothed l_x values, is given in table 3, which has been calculated from the following equation ;

$$l_x = 1.00 e^{-0.0183001x^2}, \quad (2)$$

considering the 83rd, 84th, 85th, ..., 98th day of the pivotal age as the 0th, 1st, 2nd, ..., 15th day of the age of mature females (*vide* table 2). In the present table, the l_x column presents the adult survival rate only, while m_x gives the number

Table 3. Life table and fecundity schedule for *Olenecamptus bilobus* (Fabr.) on *Artocarpus chaplasha* Roxb.

Pivotal age in days (x)	Survival of females at different age intervals (l_x)	Age specific fecundity (\varnothing births) (m_x)	($DI_x \cdot m_x$)	Actual \varnothing births per time unit (k_x)	($x \cdot k_x$)
0-77	**	..	Immature Stages		
78	1.000	*	0.441	0.441	34.398
79	1.000	*	0.441	0.441	34.839
80	1.000	*	0.441	0.441	35.280
81	1.000	*	0.441	0.441	35.721
82	1.000	*	0.441	0.441	36.162
83	1.000	2.52	1.111	1.111	92.213
84	0.982	4.31	1.901	1.867	156.828
85	0.929	5.98	2.637	2.450	208.250
86	0.848	7.05	3.109	2.636	226.696
87	0.746	7.67	3.382	2.523	219.501
88	0.633	8.73	3.691	2.336	205.568
89	0.517	7.49	3.303	1.708	152.012
90	0.408	7.06	3.113	1.270	114.300
91	0.310	6.19	2.730	0.846	76.986
92	0.227	5.68	2.505	0.569	52.348
93	0.160	5.68	2.505	0.401	37.293
94	0.109	4.59	2.024	0.221	20.774
95	0.072	4.34	1.914	0.138	13.110
96	0.045	4.07	1.795	0.081	7.776
97	0.028	3.34	1.473	0.041	3.977
98	0.016	1.08	0.476	0.008	0.784
99	0.000	0.00	0.000	0.000	0.000

Net reproductive rate = $R_0 = \sum k_x = 20.411$; $\sum x \cdot k_x = 1746.816$.

* Pre-oviposition period. ** Developmental survival rate = 0.441.

of female eggs laid by the average female per day. For presenting the results in a more convenient way, an additional column ($DI_x \cdot m_x$) has been proposed, which gives the product of the number of female eggs laid by the average female per day and the developmental survival rate. Another column gives the product of $DI_x \cdot m_x$ and the adult survival rate (l_x in the present table), whose product is designated by k_x in the present communication.

The total number of female eggs laid per female of the original cohort (R_0) has been estimated at 20.411, which indicates that 20.411 females are produced per female per generation. The maximum pre-oviposition period has been observed to be 5 days, i.e., from the 78th to 82nd day of the pivotal age, and oviposition continues almost throughout the life span of the females. Maximum contribution ($m_x = 8.37$) in the life-cycle is observed to be made by the females of the 88th

day of the pivotal age (*vide* table 3). The first female mortality within the cohort occurs on the 7th day ($l_x = 0.982$) after the emergence of the adult female and mortality increases gradually thereafter, as shown in figure 1.

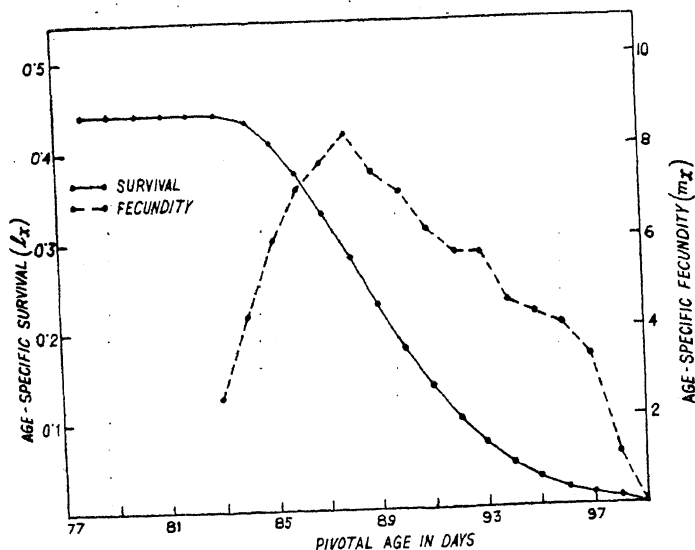


Figure 1. Age-specific survival and fecundity of *Olenecamptus bilobus* (Fabr.) on *Artocarpus chaplasha* Roxb.

Table 4. Mean length of generation, innate capacity for increase in numbers and finite rate of increase in numbers in *Olenecamptus bilobus* (Fabr.) on *Artocarpus chaplasha* Roxb.

Particulars

1. Cohort generation time (T_c)

$$T_c = \frac{\sum x \cdot k_x}{R_0} = \frac{1764.816}{20.411} = 86.464 \text{ days}$$

2. Innate capacity for increase in numbers (r_m)

$$r_m = \frac{\ln R_0}{T_c} = \frac{\log e^{R_0}}{T_c} = \frac{3.0161}{86.464} = 0.0349$$

3. Finite rate of increase in numbers

$$\begin{aligned} (\lambda) &= \text{Natural antilog of } r_m \\ &= \text{Natural antilog of } 0.0349 \end{aligned} = 1.03552$$

4. Corrected generation time (T)

$$T = \frac{\ln R_0}{r_m} = \frac{\log e^{R_0}}{r_m} = \frac{3.0161}{0.0349} = 86.42 \text{ days}$$

5. Weekly multiplication of population

$$= (e^{r_m})^7 = (1.03552)^7 = 1.2767$$

Table 5. Stable age-distribution of *Olenecamptus bilobus* (Fabr.) on *Artocarpus chaplasha* Roxb.

$$(r_m = 0.0349)$$

Pivotal age in days _a	L_x	$e^{-r_m(x+1)}$	$L_x \cdot e^{-r_m(x+1)}$	Percentage age-distribution $100 \beta L_x \cdot e^{-r_m(x+1)}$	
1	2	3	4	5	
0	1.000	0.9657	0.9657	4.6874	Total eggs 24.95%
1	1.000	0.9326	0.9326	4.5268	
2	1.000	0.9006	0.9006	4.3714	
3	1.000	0.8697	0.8697	4.2214	
4	0.930	0.8399	0.7811	3.7914	
5	0.850	0.8111	0.6894	3.3463	
6	0.850	0.7833	0.6658	3.2317	
7	0.840	0.7564	0.6354	3.0842	
8	0.830	0.7304	0.6062	2.9424	
9	0.825	0.7054	0.5820	2.8250	
10	0.820	0.6812	0.5586	2.7114	Total larvae 68.22%
11	0.815	0.6578	0.5361	2.6022	
12	0.810	0.6352	0.5145	2.4973	
13	0.805	0.6135	0.4939	2.3974	
14	0.800	0.5924	0.4739	2.3003	
15	0.800	0.5721	0.4577	2.2216	
16	0.800	0.5525	0.4420	2.1454	
17	0.795	0.5336	0.4242	2.0590	
18	0.790	0.5153	0.4071	1.9760	
19	0.785	0.4976	0.3906	1.8959	
20	0.780	0.4805	0.3748	1.8192	
21	0.765	0.4640	0.3550	1.7231	
22	0.750	0.4481	0.3361	1.6314	
23	0.745	0.4327	0.3224	1.5659	
24	0.740	0.4179	0.3092	1.5008	
25	0.735	0.4036	0.2966	1.4397	
26	0.730	0.3897	0.2845	1.3809	
27	0.725	0.3764	0.2729	1.3246	
28	0.720	0.3635	0.2617	1.2703	
29	0.710	0.3510	0.2492	1.2096	
30	0.700	0.3390	0.2373	1.1518	
31	0.695	0.3273	0.2275	1.1043	
32	0.690	0.3161	0.2181	1.0586	
33	0.690	0.3053	0.2107	1.0227	
34	0.690	0.2948	0.2034	0.9873	
35	0.680	0.2847	0.1936	0.9397	
36	0.670	0.2749	0.1842	0.8941	
37	0.665	0.2655	0.1766	0.8572	
38	0.660	0.2564	0.1692	0.8213	
39	0.650	0.2476	0.1609	0.7810	
40	0.640	0.2391	0.1530	0.7426	
41	0.630	0.2309	0.1455	0.7062	
42	0.620	0.2230	0.1383	0.6713	
43	0.615	0.2153	0.1324	0.6427	
44	0.610	0.2079	0.1268	0.6155	
45	0.610	0.2008	0.1225	0.5946	
46	0.610	0.1939	0.1183	0.5742	

1	2	3	4	5	
47	0.610	0.1873	0.1143	0.5548	
48	0.610	0.1808	0.1103	0.5354	
49	0.605	0.1746	0.1056	0.5126	
50	0.600	0.1687	0.1012	0.4912	
51	0.600	0.1629	0.0977	0.4742	
52	0.600	0.1573	0.0944	0.4582	
53	0.600	0.1519	0.0911	0.4422	
54	0.595	0.1469	0.0874	0.4242	
55	0.590	0.1416	0.0835	0.4053	
56	0.585	0.1368	0.0800	0.3883	
57	0.580	0.1321	0.0766	0.3718	
58	0.580	0.1276	0.0740	0.3592	
59	0.580	0.1232	0.0715	0.3471	
60	0.580	0.1190	0.0690	0.3349	
61	0.575	0.1149	0.0661	0.3208	
62	0.570	0.1109	0.0632	0.3068	Total pupae 4.38%
63	0.565	0.1071	0.0605	0.2937	
64	0.560	0.1035	0.0580	0.2815	
65	0.545	0.0999	0.0544	0.2641	
66	0.530	0.0965	0.0511	0.2480	
67	0.515	0.0932	0.0480	0.2330	
68	0.500	0.0900	0.0450	0.2184	
69	0.495	0.0869	0.0430	0.2087	
70	0.490	0.0839	0.0411	0.1995	
71	0.480	0.0810	0.0389	0.1988	
72	0.480	0.0781	0.0375	0.1820	
73	0.475	0.0756	0.0359	0.1743	Total dormant adults 1.16%
74	0.470	0.0730	0.0343	0.1665	
75	0.455	0.0705	0.0321	0.1558	
76	0.441	0.0681	0.0300	0.1456	
77	0.441	0.0657	0.0290	0.1408	
78	0.441	0.0635	0.0280	0.1359	
79	0.441	0.0613	0.0270	0.1311	
80	0.441	0.0592	0.0261	0.1267	
81	0.441	0.0572	0.0252	0.1223	
82	0.441	0.0552	0.0243	0.1180	
83	0.437	0.0533	0.0233	0.1131	
84	0.422	0.0515	0.0217	0.1053	
85	0.392	0.0497	0.0195	0.0947	
86	0.352	0.0480	0.0169	0.0820	
87	0.304	0.0464	0.0141	0.0684	
88	0.254	0.0448	0.0114	0.0553	
89	0.204	0.0432	0.0088	0.0427	Total adults 1.29%
90	0.156	0.0418	0.0065	0.0316	
91	0.119	0.0403	0.0048	0.0233	
92	0.086	0.0389	0.0033	0.0160	
93	0.060	0.0376	0.0023	0.0112	
94	0.040	0.0363	0.0015	0.0073	
95	0.026	0.0351	0.0009	0.0044	
96	0.016	0.0339	0.0005	0.0024	
97	0.010	0.0327	0.0003	0.0015	
98	0.004	0.0316	0.0001	0.0005	

$$1/\beta = 20.6019$$

The present investigation suggests that the innate capacity of increase (r_m) is 0.0349 per female per day, while the daily finite rate of increase (λ) is 1.03552 (table 4). The mean time for completing a generation (T) has been calculated at 86.42 days. It appears, therefore, that under a given set of conditions in the laboratory, the daily finite rate of increase ($\lambda = 1.03552$) enables the borer insect to multiply by 1.2767 times every week.

From the present observations, the contribution made by the different developmental stages of *O. bilobus* towards the stable age-distribution has also been determined. The results are presented in table 5, in which the life-table age-distribution (L_x) has been worked out with the following formula;

$$L_x = \int_x^{x+1} l_x dx,$$

which, in practice, is given by

$$L_x = \frac{l_x + (l_{x+1})}{2}. \quad (3)$$

It has been observed that, on reaching the stable age-distribution, the egg, larva, pupa, dormant adult and adult stage of this insect contribute to the extent of 24.95, 68.22, 4.38, 1.16 and 1.29% respectively.

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References

- Atwal A S and Bains S S 1974 *Applied animal ecology* (Ludhiana : Kalyani Publishers) pp. 128-135
- Birch L C 1948 The intrinsic rate of natural increase of an insect population; *J. Anim. Ecol.* 17 15-26
- Howe R W 1953 The rapid determination of intrinsic rate of increase of an insect population; *Ann. Appl. Biol.* 40 134-155
- Khan T N and Maiti P K 1980 Bionomics of the round head borer, *Olenecamptus bilobus* (Fabricius) (Coleoptera : Cerambycidae); *Proc. Zool. Soc. Calcutta* (In press)
- Laughlin R 1965 Capacity for increase : A useful population statistics; *J. Anim. Ecol.* 34 77-92
- Leslie P H and Ranson R M 1940 Mortality, fertility and rate of natural increase in the vole (*Microtus agrestis*) in the laboratory; *J. Anim. Ecol.* 9 27-52
- Pearl R and Parker S L 1924 Experimental studies on the duration of life. IX. New life tables for *Drosophila*; *Am. Nat.* 58 71-82

Behavioural responses of the Indian gerbil, *Tatera indica* to conspecific sebum odour of the ventral scent marking gland

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Abstract. Behavioural responses of the Indian gerbil, *Tatera indica*, to conspecific sebum odour of the male ventral marking scent gland were studied in a glass cage and a plus maze. Male and female gerbils were attracted towards the strange male sebum odour though its magnitude was low in females possessing the ventral marking gland, still lower in the females in which the marking gland was absent. The diversity in preferential behaviour of female *Tatera indica* is discussed in relation to the role of ventral marking behaviour in chemical communication among rodents. Correlating the results of the experiments with our field observations, it appears that the function of scent marking in *T. indica* is more of a 'familiarisation' nature to label the habitat for its own use in orientation or to signal 'home' to the marking animal.

Keywords. Chemical communication; familiarisation; gerbil; homing; phago-stimulant; scent marking; *Tatera indica*.

1. Introduction

The Indian gerbil, *Tatera indica*, a Turanian element (Prakash 1974), is distributed throughout the Indian sub-continent. The sub-species *T. i. indica* is one of the predominant rodents found in the desert region, occupying almost all the habitat types (Prakash 1975). Whereas a number of gerbil families inhabit a single burrow in the village complex or around an urban area, they live individually or in pairs in open desert grasslands. These two distinct types of *Tatera* populations exhibit a difference in the intensity of occurrence of the mid-ventral scent marking gland in females. In the former population in which social organisation is intense, 5% adult females possess it whereas in the scattered populations it is present in 12% of the females. However the gland occurs in about 89% adult males in both the types of populations. The difference in the frequency of occurrence of scent-marking gland in female *Tatera* existing in two types of social organisation is being reported for the first time among rodents and it makes the functional role of this gland in chemical communication more intricate as well as interesting. The results of our experiments to investigate the behavioural

responses of male and female Indian gerbil, *Tatera indica indica* towards the sebum (secretion of the ventral marking scent gland) odour are presented in this communication.

2. Methods

All the gerbils, *T. i. indica* were collected from the sandy habitat around Jodhpur 26° 18' N, 73° 01' E). The first experiment was conducted in a glass cage (90 × 30 × 30 cm). 60 gerbils (30 ♂, Avg. body weight 129.53 ± 5.1 g and 30 ♀, 110.4 ± 4.5 g) were individually released in the cage one by one and were oriented for 6 days and then exposed to sebum odour of a strange male. Before releasing the next gerbil, the cage was thoroughly washed and dried. One glass slide smeared with the sebum of a strange male was placed on one side of the cage and another clean slide on the other side, following Kumari and Prakash (1981a), to avoid new object reaction (Mathur and Prakash 1980). The experimental gerbil was released in the middle of the cage and its behaviour (sniffing, licking, urination, defecation, ventral marking) in relation to the individual stimulus on both the sides of the cage was observed for 30 min. The number of visits and duration of every behavioural act in the vicinity of the two slides were recorded with the help of a stop watch. Observations were made at night under infra-red light at the maximum activity epoch of the gerbils.

In the second experiment, the behavioural responses of *T. indica* were observed in a residential plus maze (Bhardwaj and Prakash 1981), both by ocular observations and by recording the relative food consumption. 10 male (Av. body weight 120.7 ± 8.2 g) and 10 female (96.0 ± 7.9 g) were released one by one in the plus maze and were acclimatized for 6 days in the new environment. Thereafter, the gerbils were provided weighed quantity of pearl millet (*Pennisetum typhoides*) in arms A and C, drinking water in arm D. The arm B remained empty. 24-hour consumption of millet in both the food baskets was recorded for 6 days. Whereafter, a slide carrying sebum smear of a strange male (*T. indica*) was placed in the arm in which food consumption was lower (A), and a blank slide in the other arm (C) near the food container. Food consumption was again recorded in both the arms for 4 days. Rodents were released in the central chamber of the plus maze and were free to move and explore any of the arms. A complete record of their visits and duration to every arm was maintained for 15 min soon after the introduction of the two slides.

3. Results

A comparison of the mean number of visits by gerbils to the sebum odour slide and clean slide in the glass cage indicates that the frequency of visits to each section was similar but the duration of visits was significantly more in the side in which the sebum odour slide was lodged (students *t*, $P < 0.001$, Bailey 1959, table 1). Male as well as female gerbils were attracted towards the sebum odour of strange male though its magnitude was lower among females as indicated by their behavioural responses (table 2).

Table 1. Olfactory response of *Tatera indica* to conspecific male sebum odour.

Stimulus		No. of visits per 30 min Mean \pm SE	Duration of response (seconds) Mean \pm SE
Male	Male sebum	27.10 \pm 1.85	17.60 \pm 0.87***
	Blank slide	27.16 \pm 1.87	12.41 \pm 0.64
Female	Male sebum	31.13 \pm 1.93	16.70 \pm 0.38***
	Blank slide	30.90 \pm 1.91	13.72 \pm 0.50

Level of significance (Student's *t* test, Bailey 1959).

*** = $P < 0.001$.

Male as well as female (possessing the ventral marking scent gland) *T. indica* sniffed ($P < 0.001$), urinated ($P < 0.05$) significantly more times (table 2) in presence of another male's sebum odour. Male gerbils significantly ventral marked ($P < 0.001$) and licked ($P < 0.05$) more in the cage side carrying the odour stimulus. No difference was, however, observed in grooming behaviour. The females, however, did not differ in their response to strange male sebum odour in respect of various social acts (table 2).

In the plus maze, after the sebum smeared slide was placed near the food basket in the arm (A) in which consumption of plain pearl millet was significantly ($P < 0.001$) lower than in arm C (table 3), the food consumption by both male and female *Tatera indica* in the former increased significantly ($P < 0.001$). However, in case of males, in the presence of the two set of slides (columns 3 and 4, table 3) millet intake by male gerbils declined significantly ($P < 0.05$) in arm C but in case of females this difference was not statistically significant which indicates that the attraction towards strange male sebum by the females is of lower intensity as compared to that by males.

Both male and female *T. indica* indicated a similar behavioural pattern, as in the glass cage, by showing an increased frequency of sniffing and licking ($P < 0.01$) in the section of sebum smeared slide (figure 1). The frequency of ventral marking activity by males also exhibited an enhanced rate ($P < 0.01$) towards the sebum slide but the females did not ventral mark at all because these were the females which had no ventral scent marking gland (figure 1). A similar pattern of the duration of various social acts was also observed.

4. Discussion

The ventral scent marking gland is present in both the sexes in the genus *Meriones* (Sokolov and Skurat 1966; Kumari *et al* 1981) but in the genus *Tatera* in which the occurrence of such a gland was reported earlier (Prakash and Kumari 1979), it is present in 89% males and only in a few females. Results of our experiments

Table 2. Mean number and duration (seconds) \pm SE of behavioural acts by *Tatera indica* per 30 min in presence of conspecific male sebum odour and blank slide.

Sex	Stimuli	Sniffing		Licking		Ventral marking		Grooming		Urination		Defecation	
		N	D	N	D	N	D	N	D	N	D	N	D
Male	Sebum smear	2.5*** ± 0.34	10.1*** ± 1.61	1.63* ± 0.33	7.70** ± 1.79	4.00*** ± 0.64	6.68** ± 0.64	2.03NS ± 0.29	59.83NS ± 5.87	1.46** ± 0.23	1.43NS ± 0.20		
	Blank slide	1.30 ± 0.22	5.42 ± 1.04	0.84 ± 0.21	3.20 ± 0.82	1.96 ± 0.40	4.01 ± 0.51	2.00 ± 0.46	33.43 ± 7.88	0.86 ± 0.09	0.86 ± 0.23		
Female	Sebum smear	2.53** ± 0.40	11.00*** ± 1.79	1.13NS ± 0.28	6.73NS ± 1.75	1.30NS ± 0.38	2.15NS ± 0.64	1.36NS ± 0.27	21.26NS ± 3.25	1.03** ± 0.20	1.23NS ± 0.27		
	Blank slide	1.30 ± 0.36	4.75 ± 0.96	0.70 ± 0.18	3.50 ± 1.20	0.76 ± 0.35	1.38 ± 0.60	0.96 ± 0.24	23.73 ± 7.63	0.43 ± 0.12	0.96 ± 0.20		

N = Number of act, D = Duration in seconds

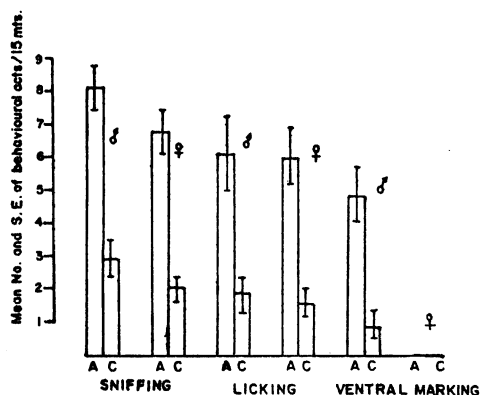
Level of significance NS = Not significant * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Table 3. Food consumption by *Tatera indica* in absence and presence of conspecific male sebum odour.

Sex	Mean food consumption: g/100 g body weight \pm SE			
	Arm A	Arm C	Arm A	Arm C
	(without any stimulus)	(without any stimulus)	(with sebum odour slide)	(with plain slide)
	1	2	3	4
Male	1.68 \pm 0.37	3.25 \pm 0.38	3.58 \pm 0.45	2.46 \pm 0.37
Female	1.80 \pm 0.31	3.75 \pm 0.42	3.90 \pm 0.36	3.28 \pm 0.42

Level of significance between

- 1 and 3 male < 0.001
- 1 and 3 female < 0.001
- 2 and 4 male Not significant
- 2 and 4 female Not significant
- 3 and 4 male < 0.05
- 3 and 4 female Not significant.

**Figure 1.** Frequency of various behavioural acts by male and female (not possessing the ventral scent-marking gland) *T. indica* in the two arms of plus maze, one carrying strange male sebum odour (A) and the other without it (C).

clearly indicate that all the three categories of *T. indica* (males possessing scent marking gland), females possessing the gland (experiment 1 in glass cage) and females without them (experiment 2 in plus maze) are attracted towards the sebum odour of strange male suggesting that it has a bio-chemical communication function in this genus. However, the frequency and duration of social acts by male and female *T. indica* clearly indicate that the magnitude of attraction towards strange male odour is much lower among females as compared to males. In this preferential behaviour *T. indica* is similar to *Meriones tristrami* (Thiessen *et al* 1973)

and *M. unguiculatus* (Thiessen *et al* 1970). Indian desert gerbil, *M. hurrianæ* prefers the gland odour of similar sex given the choice of odour of both sexes. However, in the absence of the same sex odour, both male and female are attracted towards opposite sex odour (Kumari and Prakash 1981a). In this behaviour the females of *T. indica* differ from *M. hurrianæ* though they occur in similar habitats in the Indian desert and are the two most predominant rodent species of the region.

We have already reported that if the food is impregnated with sebum odour it functions as phago-stimulant in *T. indica* (Kumari and Prakash 1979). This observation is further confirmed by the results of experiment 2 in as much as that even when the experimental females were those which did not possess the scent gland, their food consumption increased significantly ($P < 0.001$) in the presence of the sebum odour (table 3).

Another interesting observation made is about the significant ($P < 0.001$; table 2) enhancement of ventral marking activity by male and females (possessing the scent-marking gland) in the presence of the sebum odour of a strange male. In the plus maze experiments, similar enhancement ($P < 0.01$) in marking behaviour of males was observed but this social behaviour in females was entirely absent. These were the females in which the scent gland was absent and as such the social act of ventral marking was not expected. What is interesting is that even in the absence of the gland they were attracted towards male sebum odour (table 2). The variance in the ventral marking behaviour of the two groups of females but the similarity of their being attracted towards strange male sebum odour complicate the functional role of ventral scent-marking gland.

A number of workers have explained the functions of ventral scent marking among rodents. Ewer (1968) stated that an animal's own scent might act to "increase its confidence" in the environment, whereas Eibl-Eibesfeldt (1953) and Mykytowycz (1968) conjecture that scent marks provide "homeliness" to the animals. Other functions designated to scent marking are territorial (Thiessen 1973), individual identification (Daly 1977), recognition of pups (Wallace *et al* 1973), phago-stimulant (Kumari and Prakash 1979), food reservation (Kumari and Prakash 1981a) and "advertising ready to mate" stage by estrous females (Kumari and Prakash 1981b). On the basis of limited observations on *T. indica* it is rather difficult to delineate the exact function of ventral marking and the role of sebum odour. However, our observations (unpublished) in a large enclosure, the Rattery, under infra-red light show that *T. indica* ventral mark their burrow openings quite often, more so before entering a burrow opening. It was also noted that scent marking is carried out at an enhanced frequency by the chasing *Tatera*, soon after the chased one enters a burrow. *Tatera* also mark the food containers, the grass clumps and any new object whether it is a stone or a wooden peg. From these observations, it appears that the function of scent marking in *T. indica* is more of a "familiarisation" nature, or to signal 'home' to the marking animal or that of labelling the habitat for an animal's own use in orientation (Johnson 1973). Whether it plays any territorial role is not clear. Besides, these animals perform an anal drag behaviour and leave a slightly moist surface, they possibly mark with urine or with a pheromone contained in it. It is now known that the urine of *Tatera indica* has a phago-stimulant property

(Kumari and Prakash, unpublished data). These odours may leave sufficient olfactory cues which might deter other conspecifics away from the occupied territory. Our observations suggest that marking behaviour can have a number of functions and more intensive work, which is in progress, may reveal the secrecy of scent marking in rodents and its role in the bio-chemical communication.

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References

- Bailey N T J 1959 *Statistical methods in biology* (London: The English University Press)
- Bhardwaj D and Prakash I 1981 Movements of *Rattus rattus* in an artificial environment; *Indian J. Exp. Biol.* **19** 794-796
- Daly M 1977 Some experimental tests of the functional significance of scent marking by gerbils (*Meriones unguiculatus*); *J. Comp. Physiol. Psychol.* **91** 1082-1094
- Eibl-Eibesfeldt I 1953 Zur Ethologie des Hamsters (*Cricetus cricetus* L.); *Z. Tierpsychol.* **10** 204-254
- Ewer R F 1968 *Ethology of mammals* (London: Logos Press)
- Johnson R P 1973 Scent marking in mammals; *Anim. Behav.* **21** 521-535
- Kumari S, Cowan P and Prakash I 1981 The mid-ventral gland of the Indian desert gerbil; *Acta Theriol.* **26** 97-106
- Kumari S and Prakash I 1979 Conspecific odour as phagostimulant for Indian gerbil, *Tatera indica* Hardwicke; *Indian J. Exp. Biol.* **17** 981-982
- Kumari S and Prakash I 1981a Behavioural responses of *Meriones hurrianae* (Jerdon) in conspecific sebum odour of ventral sebaceous gland; *Biol. Behav.* **6** 255-264
- Kumari S and Prakash I 1981b Scent marking behaviour of *Meriones hurrianae* during oestrous; *Anim. Behav.* **29** 1269-71
- Mathur R P and Prakash I 1980 New food reaction among desert rodents; *Saugetier Mitteil.* **28** 28-30
- Mykityowycz R 1968 Territorial marking by rabbit, *Sci. Am.* **218** 116-126
- Prakash I 1974 The Ecology of vertebrates of the Indian desert. Chapter XIII in *Biogeography and ecology in India* (eds.) M S Mani, Dr Junk (The Hague: b.v. Verlag) pp. 369-420
- Prakash I 1975 The ecology and zoogeography of mammals. Chapter XIX in *Environmental analysis of the Thar desert* (eds.) R K Gupta and Ishwar Prakash (Dehradun: English Book Depot) 468-480
- Prakash I and Kumari S 1979 Occurrence of the ventral marking gland in Indian desert rodents; *Saugetier. Mitteil.* **27** 315-316
- Sokolov W and Skurat L 1966 A specific mid-ventral gland in gerbils; *Nature (London)* **211** 544-545
- Thiessen D D 1973 Footholds for survival; *Am. Sci.* **61** 346-351
- Thiessen D D, Lindzey G, Blum S L and Wallace P 1970 Social interactions and scent marking in the Mongolian gerbil (*Meriones unguiculatus*); *Anim. Behav.* **19** 505-513
- Thiessen D D, Wallace P and Yahr P 1973 Comparative studies of glandular scent marking in *Meriones tristrami* an Israeli gerbil; *Horm. Behav.* **4** 143-147
- Wallace P, Owen K and Thiessen D D 1973 The control and function of maternal scent marking in the Mongolian gerbil (*Meriones unguiculatus*); *Physiol. Behav.* **10** 463-466

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Effect of temperature and humidity on the development and fertility-fecundity of *Acrida exaltata* Walk.

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Abstract. The effect of temperature and humidity on *Acrida exaltata* Walk. has been studied to have two aspects in relation to (i) its effect on hopper development and (ii) its effects on the fertility-fecundity. The rate of development was affected by the moisture present in the environment. No development took place at 0%, 10% and 20% RH. Most desirable range of humidity was between 50-70% RH. Development took place at 20°, 30°, 35° and 40° C in 92.8, 74.8, 71.8 and 66.6 days in males and 101.0, 86.4, 85.6 and 75.2 days in females. Percentage of hoppers reaching the adult stage, longevity of adults, average number of copulation and average number of eggpods per female was influenced by the temperature.

Keywords. Relative humidity ; fertility-fecundity ; development ; copulation ; longevity ; temperature.

1. Introduction

Food consumption was dependent upon the relative humidity (RH) present in the atmosphere and water content in the food (Sanger 1973). Water content of the eggpods directly affects the size of hatchlings in *Schistocerca gregaria*, water loss during the last half of incubation period resulting in smaller hatchlings and excess water uptake in larger hatchlings (Bernays 1972). Shulov (1970) in *Nomadacris septemfasciata* and *Locusta migratoria* found that humidity and temperature affects the development and weight of eggs. Development was arrested when the required moisture was not available, and resumed on being provided with moisture. Petty (1974) in *Locusta migratoria* also found that moisture affects development and hatchling. During developmental period, nymphs preferred higher relative humidity in the field as observed by Riegert (1959). Mathée (1954) observed in the case of *Locusta pardalina* that viability of eggs was dependent upon the high temperature of the soil. According to Symmons *et al* (1974), temperature range between 30°-40° C was favourable for development. Abou-Elela and Hilmy (1977), Tutkun (1973) and Qayyum and Atique (1973) found that temperature directly influences hatching of eggs, rate of develop-

ment, precopulation, preoviposition period and reproduction. Studies were made to note the effect of temperature and humidity on the hoppers development and fertility-fecundity of *Acrida exaltata* Walk.

2. Material and methods

2.1. Effect of humidity on hoppers development and fertility-fecundity

Newly hatched hoppers were kept in jars and glass tubes at 90, 80, 70, 50, 20, 10 and 0% humidity at a constant temperature of 37° C for observations on nymphal duration and fertility-fecundity. Desired relative humidity was obtained through the solution of potassium hydroxide (100 gm of KOH per 100 gm of water) as given by Buxton and Mellanby (1934) (table 1).

2.2. Effect of temperature on hoppers development and fertility-fecundity

Newly hatched hoppers were kept individually in glass tubes (15 × 3.8 cm) covered with muslin cloth for observation on hoppers development at 25°, 30°, 35° and 37° C. Nymphs were provided daily with fresh food twice. Standard error was worked out and results are shown in table 2.

Newly emerged adults were kept in pairs at temperature of 20°, 25°, 30°, 35° and 37° C in separate glass jars. Number of copulations and ovipositions was observed and number of eggpods per female and longevity of adults were noted. Results are summarized in table 3.

3. Observations

3.1. Effect of humidity on the hopper development and fertility-fecundity

Rate of development of hoppers was affected by moisture present in the environment. No development took place at 0%, 10%, and 20% RH (table 4). The most desirable range of humidity was between 50-70% RH. Per cent-

Table 1. Relative humidity obtained through KOH with differing specific gravity.

RH %	gm KOH/100 gm of water	Specific gravity
90	15.0	1.115
80	25.0	1.175
70	35.0	1.265
50	52.0	1.335
20	87.5	1.490
10	110.0	1.570
0	Solid KOH	..

Table 2. Effect of temperature on the hoppers development.

Stage		25° C (days)		30° C (days)		35° C (days)		37° C (days)	
		♂	♀	♂	♀	♂	♀	♂	♀
I Instar	Mean	14.40	15.60	11.20	13.80	7.80	9.20	7.20	8.00
	SE	0.81	0.93	0.66	0.86	0.70	0.58	0.37	0.45
II Instar	Mean	14.80	17.80	13.60	12.20	7.60	7.80	6.60	7.20
	SE	0.66	0.86	1.03	0.66	0.61	0.66	0.40	0.58
III Instar	Mean	13.40	15.80	10.80	11.80	8.40	10.40	7.40	8.80
	SE	0.87	0.86	0.58	0.86	0.76	0.68	0.51	0.58
IV Instar	Mean	12.80	15.80	13.80	13.60	9.80	12.20	8.60	10.20
	SE	0.58	0.58	0.86	0.51	0.58	0.66	0.60	0.58
V Instar	Mean	13.60	16.40	12.80	15.80	9.20	11.20	8.40	10.20
	SE	0.51	0.76	0.58	0.80	0.37	0.37	0.51	0.58
VI Instar	Mean	14.80	17.20	14.20	17.40	14.20	16.20	13.40	14.40
	SE	0.37	0.66	0.86	1.03	0.37	0.66	0.60	0.60
VII Instar	Mean	14.20	17.60	14.20	17.00	15.40	18.60	14.00	16.40
	SE	0.37	0.51	0.58	0.74	0.68	1.03	0.55	1.03
Adult	Mean	92.80	101.00	74.80	86.40	71.80	85.60	66.60	75.20
	SE	2.73	2.57	2.17	1.72	0.98	3.02	1.91	2.35
Percentage of hoppers reached adult stage		34.20	31.00	51.50	47.20	73.50	68.00	76.00	71.50

SE = Standard error.

tage of hoppers reaching the adult stage was the highest, 77.86 and 81.25 in males and females respectively at 70% RH, while the lowest, 30.75 and 43.0 in males and females respectively at 90% RH.

As evident from table 2, it was found that at 70% RH, sexual maturation is hastened, but the longevity of adults was shorter, 87 and 116 days in males and females respectively due to the rapid rate of sexual maturation and a high average number of eggs and eggpods per female. As the humidity increased above the optimum (70% RH) the longevity of adults and the time required for sexual maturation increased, but the number of eggs and eggpods per female decreased until 80% RH. Above 80% RH the length of adult life decreased and the number of eggpods were few or none at all. The number of eggpods per female and the number of eggs per pod show a rapid drop at relative humidities below 70% RH.

Table 3. Effect of temperature on fertility-fecundity and longevity of *Acrida exaltata* Walk.

Temperature °C	Sex	No. of pairs	Total mating	Longevity of adults (days) Mean \pm SE	Average no. of copula- tion per male	Average no. of egg pods per female	Average no. of eggs per pod
20	Male	5	8	68 \pm 2.23	1.6	1	48
	Female			99 \pm 2.82			
25	Male	5	14	72 \pm 0.55	2.8	2	59
	Female			104 \pm 1.57			
30	Male	5	21	78 \pm 1.39	4.2	4	76
	Female			113 \pm 1.93			
35	Male	5	26	91 \pm 1.56	5.2	5	81
	Female			121 \pm 2.82			
37	Male	5	28	87 \pm 1.59	5.6	7	87
	Female			116 \pm 2.95			

SE = Standard error.

3.2. Effect of temperature on the hoppers development and fertility-fecundity

Temperature has a marked influence on the development of hoppers. Rate of development increased at the higher temperatures while at low temperature decreased as is clearly evident from table 2. The total number of mating was increased with the rise in temperature. As shown in table 3, the frequency of copulation, number of egg pods per female and number of eggs per pod increased with the rise in temperature. The temperature range between 30°-37° C was found favourable for copulation, oviposition and number of egg pods per female. Pre-copulation and pre-oviposition period decreased with rise in temperature. Average survival of adults at 35° C was higher than at 25° C. Longevity of adults was 91 and 121 days at 35° C while at 25° C, it was 68 and 99 days in males and females respectively.

4. Discussion

Effect of temperature and humidity on hoppers development must be discussed together, because it is very difficult in experimental work to separate the two factors. On the one hand, the relative humidity of the air varies with temperature, and on the other, hopper metabolism is possibly more affected by the water content of food than by air humidity, both of which may influence the

Table 4. Effect of humidity on hoppers development.

Stage		50% RH		70% RH		80% RH		90% RH		
		♂	♀	♂	♀	♂	♀	♂	♀	
		(days)		(days)		(days)		(days)		
I	Instar	Mean	8.00	9.80	7.20	8.00	9.80	12.40	15.20	19.20
		SE	0.70	0.73	0.37	0.45	0.73	0.93	0.86	1.28
II	Instar	Mean	8.80	10.40	6.60	7.20	11.40	13.20	17.00	20.20
		SE	0.66	0.87	0.40	0.58	0.92	1.06	0.70	1.39
III	Instar	Mean	8.40	11.20	7.40	8.80	11.20	12.20	16.40	18.60
		SE	0.50	1.06	0.51	0.58	0.66	0.66	0.76	1.07
IV	Instar	Mean	8.40	11.00	8.60	10.20	13.00	13.80	15.60	19.80
		SE	0.76	0.89	0.60	0.58	1.14	1.06	1.07	1.06
V	Instar	Mean	9.20	11.20	8.40	10.20	12.80	15.60	18.20	22.20
		SE	0.37	0.66	0.51	0.58	0.58	0.81	0.86	1.24
VI	Instar	Mean	12.20	15.60	13.40	14.40	12.80	15.80	16.80	20.80
		SE	0.58	0.92	0.60	0.60	0.86	0.80	0.66	1.42
VII	Instar	Mean	13.80	15.80	14.00	16.40	14.80	16.20	15.00	18.80
		SE	0.66	0.66	0.55	1.03	0.86	0.86	1.04	1.15
Adult		Mean	69.00	82.00	66.60	75.00	86.60	91.60	111.60	124.60
		SE	2.22	2.21	1.91	2.35	2.01	2.71	2.35	2.29
Percentage of nymphs reaching adult stage			71.50	73.25	77.86	81.25	58.00	61.25	38.75	43.00

SE = Standard error.

quantity of food consumed and, therefore, the rate of growth. Nevertheless, some evidence of temperature effects should be briefly mentioned. Parker (1930) in his extensive experiments with several American grasshoppers clearly indicated a shortening of hopper period and an accelerated rate of development with rising temperature.

At constant temperature, relative humidity affects the rate of ovarian growth and percentage of hoppers reaching adult stage. These variations in humidity at constant temperature suggest that the different optimal relative humidities and the lower limits are explained by different rates of evaporation (Gunn 1933; Koidsumi 1934) at different temperatures. Zolotarvesky (1933) observed in *Schistocerca gregaria* Forsk. that relative humidity plays a very important role in the embryonic and postembryonic development of locust and grasshopper. The

Table 5. Effect of humidity on fertility-fecundity of *Acrida exaltata* Walk.

Humidity % RH	No. of pairs	Sex	Longevity of adults \pm SE	Total mating	Average no. of copula- tion per male	Average no. of eggpods per female	Average no. of eggs per pod	Hatching %
10	5		No pairing and egg-laying					
20	5		No pairing and egg-laying					
50	5	Male	82.4 \pm 1.36	25	4.8	5.2	76	71.86
		Female	111.20 \pm 0.86					
70	5	Male	87.00 \pm 1.56	28	5.6	7.1	85	84.20
		Female	116.00 \pm 2.95					
80	5	Male	75.40 \pm 1.99	23	3.9	2.6	69	58.40
		Female	105.20 \pm 2.23					
90	5	Male	67.40 \pm 1.02	14	1.8	1.5	51	21.90
		Female	95.40 \pm 1.86					

SE = Standard error.

present findings are contrary to the observations of Husain *et al* (1941) and Chauvin (1941), that relative humidity has no effect on the development of hoppers. However, on examining their results, it was found that they had based their findings on experiments with only a very small number (often only one) of hoppers, and without controlling the moisture of the food. In very detailed and extensive experimental studies on locust by Hamilton (1936, 1950), the effects of a series of combinations of temperatures and relative humidities were studied and it was suggested that the duration of adult life was the shortest when conditions were optimum for sexual maturation and that the length of life gradually increased as conditions became less favourable. In the present observations 70% RH is the optimum for development and sexual maturation. As the relative humidity increased above or decreased below, the time required to reach sexual maturity was increased, but the number of eggpods decreased. This shows that with an increase in the average preoviposition period, an increase in the length of adult life is observed. Similar observations were found by Symmons *et al* (1974) in *Schistocerca gregaria*, that the temperature range between 30°-40° C was favourable for development. Abou-Elela and Hilmy (1977) observed in the case of *Acrotylus insubricus* that temperature has a direct effect on the hatching period and development.

Acknowledgements

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References

- Abou-Elela R and Hilmy N 1977 Wirkungen der Fotoperiode und Temperatur auf die Entwicklungsstadien von *Acrotylus insubricus* Scop (Orthoptera: Acrididae); *Anz. Schädlingssk. Pflanzenschutz, Umweltschutz* 50 25-28
- Bernays E A 1972 Some factors affecting size in first instar larvae of *Schistocerca gregaria* (Forsk.); *Acrida* 1 189-195
- Buxton P A and Mellanby K 1934 The measurement and control of humidity; *Bull. Entomol. Res.* 25 171-175
- Chauvin R 1941 Contribution à l'étude physiologique du criquet pèlerin et du déterminisme des phénomènes gregaires; *Ann. Soc. Entomol. Fr.* 110 133-272
- Gunn D L 1933 The temperature and humidity relations of the cockroach (*Blatta orientalis*). I. Desiccation; *J. Exp.* 10 274-285
- Hamilton A G 1936 The relation of humidity and temperature to the development of three species of African Locusts, *Locusta migratoria migratorioides* (R. and F.), *Schistocerca gregaria* (Forsk.), *Nomadacris septemfasciata* (Serv); *Trans. R. Entomol. Soc.* 85 17-60
- Hamilton A G 1950 Further studies on the relation of humidity and temperature to the development of two species of African locusts *Locusta migratoria migratorioides* (R. and F.) and *Schistocerca gregaria* (Forsk.). *Trans. R. Entomol. Soc.* 101 1-581
- Husain M A, Ahmad T and Mathur C B 1941 Studies on *Schistocerca gregaria* Forsk.—Role of water in the bionomics of the Desert Locust; *Indian J. Agric. Sci.* 10 927-944
- Koidsumi K 1934 Experimentelle studien über die transpiration und den wärmehaushalt bei insekten; *Mem. Fac. Sci. Agric. Tohoku* 12 1-179
- Mathé J J 1954 The effect of constant high temperature on the embryonic development and pulsation of the lateral body wall in *Locustana pardalina* Walk. (Orthoptera: Acrididae); *J. Ent. Soc. S. Afr.* 17 222-231
- Parker J R 1930 Some effects of temperature and moisture upon *Melanoplus mexicanus mexicanus* Sauss and *Camnula pellucida* Scudd. (Orthoptera); *Bull. Mont. Agric. Exp. Sta. no.* 223 132
- Petty G J 1974 Effect of humidity on the hatching of eggs of the brown locust, *Locusta pardalina* Walk.; *Phytophylactica* 6 305-306
- Qayyum H A and Atique M R 1973 Some ecological studies on *Chrotogonus trachypterus* Blanch; *Pakistan J. Zool.* 5 75-78
- Riegert P W 1959 Humidity reactions of *Melanoplus bivittatus* Say and *Camnula pellucida* Scudd. (Orthoptera: Acrididae); reactions of normal grasshoppers; *Can. Entomol.* 91 35-40
- Sanger K 1973 Consumption by some field grasshoppers (Orthoptera: Acrididae) depending on different humidity rates; *Verhand. Zool. Bot. Ges. Wien.* 113 81-92
- Shulov A 1970 The development of eggs of the red locust, *Numadacris septemfasciata* Serv. and the African migratory locust, *Locusta migratoria migratorioides* R and F and its interruption under particular conditions of humidity; *Anti Locust Bull.* 48 1-26
- Symmons P M, Green S M, Robertson R A and Wardhough K G 1974 The production of distribution maps of the incubation and development periods of the Desert Locust, *Schistocerca gregaria* Forsk. (Orthoptera: Acrididae); *Bull. Entomol. Res.* 64 443-451
- Tutkun F 1973 Investigation on the effect of low temperature on the last immature adult stage of the Desert Locust, *Schistocerca gregaria* Forsk.; *Bitki Koruma Bull.* 13 181-201
- Zolotarvesky B N 1933 Contribution à étude biologique du criquet migrateur, *Locusta migratoria acapito* Sauss dans ses foyers permanent; *Ann. Epiphytes* 19 47-142

On some blood flukes (Spirorchiidae : Coeuritreminae) from freshwater chelonians in India

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Abstract. *Coeuritrema sutlejensis* Mehrotra, 1973 and *C. sheilae* are described in detail and their validity is discussed. A key to the species of *Coeuritrema* is provided and a few of the diagnostic features of the genus are emended.

C. lyssimus Mehra, 1933 is recorded from a new locality and some variations from the original description are mentioned.

Keywords. Blood flukes; chelonians; *Coeuritrema*; Spirorchiidae.

During the period from January 1969 to September 1971, 156 specimens of freshwater chelonians, namely *Kachuga tectum tectum* (29), *K.t. tentoria* (5), *K. sylhetensis* (69) and *Lissemys punctata punctata* (53) from different localities in Punjab, Haryana and Uttar Pradesh were dissected for the collection of the digenetic flukes. A thorough examination of heart and blood of the hosts revealed the presence of many flukes. Of these, detailed accounts of two species *Coeuritrema sutlejensis* Mehrotra, 1973 and *C. sheilae* Mehrotra, 1973 are given here and their validity is discussed. Earlier, only the diagnostic features of these species had been given in an abstract form (Mehrotra 1973). Another species, *Coeuritrema lyssimus* Mehra, 1933, has also been recorded.

Bouin was used as a fixative for the parasites. The flattened flukes were stained with Mayer's carmalum, borax carmine or Ehrlich's haematoxylin. The last-mentioned stain and eosin were used to stain the serial sections cut at a thickness of 5 μ .

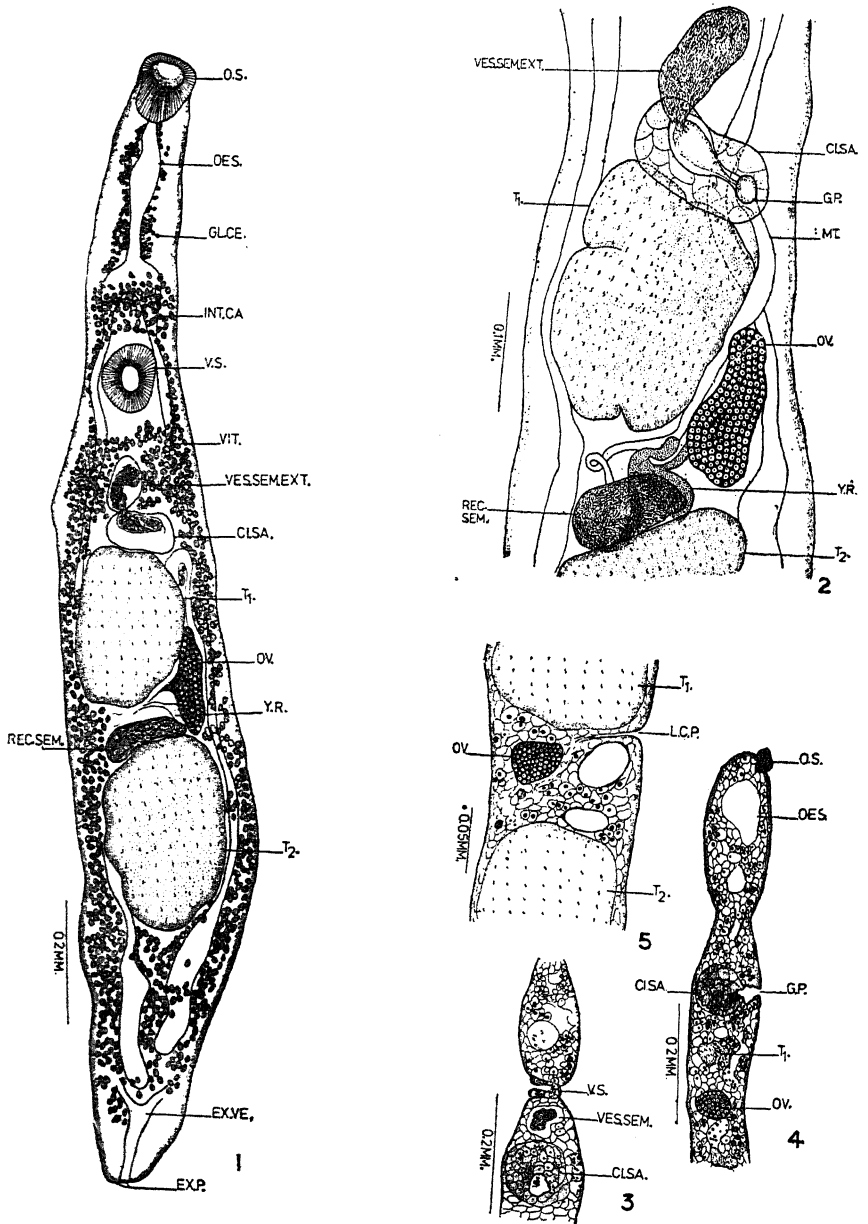
Family Spirorchiidae Stunkard, 1921

Subfamily Coeuritreminae Srivastava, 1960*

Genus *Coeuritrema* Mehra, 1933

Coeuritrema sutlejensis Mehrotra, 1973 (figures 1-5)

* Yamaguti (1971) has mentioned the subfamily name Cocuritrematidae Dwivedi, 1968. In fact Srivastava (1960), not Dwivedi, had proposed this subfamily name in view of the priority of the genus *Coeuritrema* over *Tremarhynchus*. Dwivedi (1967) has only referred to Srivastava's (1960) views.



Figures 1-5. *Coeuritrema sutlejensis* Mehrotra, 1973 1. a whole mount (ventral view); 2. a portion from another specimen showing the terminal genital ducts and other reproductive organs (magnified); 3. a portion of sagittal section showing the depth of the ventral sucker; 4. genital pore and cirrus sac in sagittal section; 5. a portion of sagittal section showing the Laurer's canal pore.

Thirteen specimens of *Coeuritrema sutlejensis* Mehrotra, 1973 were recovered from the ventricle of the heart of freshwater chelonians, *Kachuga sylhetensis* (Jerdon) collected from the River Sutlej at Ropar (Punjab) and *Lissemys punctata punctata* Bonnaterra procured from Lucknow (U.P.) and Sangrur (Punjab). The number of flukes in one host was one or two.

Description (based on 10 specimens ; all the measurements are in mm) : Body slightly tapering towards extremities, ending in blunt rounded tips, 1.32–2.15 in length by 0.22–0.40 in maximum breadth across testes. Tegument smooth, suckers prominent but with weak musculature ; oral sucker terminal, 0.08–0.12 × 0.06–0.11, ventral sucker situated almost at level of middle of anterior half of body, 0.09–0.12 × 0.08–0.13, almost equal to oral sucker. Oesophagus 0.16–0.31 long, irregularly distended and surrounded by gland cells. Intestinal caeca at first forming broad dilated shoulders and then extending backward as slender tubes, dilating again in post-testicular region and terminating asymmetrically a little in front of rear end of body.

Excretory vesicle Y-shaped with a short stem ; excretory pore terminal.

Testes enormously developed, tandem, intercaecal, with a wavy contour, anterior testis 0.17–0.27 × 0.14–0.29 and posterior testis 0.19–0.33 × 0.13–0.26. Vesicula seminalis externa a little posterior to ventral sucker, thin-walled 0.09–0.15 × 0.04–0.08. Cirrus sac almost transversely situated in between vesicula seminalis externa and anterior testis, 0.04–0.08 × 0.09–0.15. Genital pore dorsal, to left of median line, close and external to left intestinal caecum, in front or at level of anterior border of anterior testis.

Ovary sinistral, intertesticular, close to left intestinal caecum, elongated, 0.06–0.19 × 0.016–0.05. Receptaculum seminis median, intertesticular. Laurer's canal present. Uterus short, running along left margin of anterior testis. Eggs not observed in any specimen. Vitellaria extending laterally from level of intestinal bifurcation up to close behind caecal termination, coalescing in the regions just in front and behind ventral sucker and also in post-testicular zone ; in some specimens, however, the vitelline follicles have been found scattered in the region in front of the intestinal bifurcation. Yolk reservoir dorsal to receptaculum seminis.

Remarks : So far, seven species have been assigned to the genus *Coeuritrema* Mehra, 1933. These are *C. lyssimus* Mehra, 1933 from *Lissemys punctata* in Allahabad (U.P.) ; *C. odhnerensis* Mehra, 1933 from the same host and locality ; *C. indicus* (Thapar 1933) Mehra 1934 (syn. *Tremarhynchus indicus* (Thapar 1933) from *Trionyx gangeticus* in Lucknow (U.P.) ; *C. yoshidai* (Ozaki 1939) Takeuti 1942 (syn. *Hapalorhynchus yoshidai* Ozaki 1939) from *Ocadisinensis* in China ; *C. ocadiae* Takeuti 1942 from *Ocadia sinensis* in Formosa ; *C. oschmarini* Belous 1963 from *Amyda sinensis* from the Khanka lake and the River Mo in the far east of the USSR ; and *C. macrotesticularis* Rodhe, Lee et Lim, 1968 from *Dogania subplana* in Malaya.

C. sutlejensis can be distinguished from *C. lyssimus* in which the body surface is covered with conical tubercles, the cirrus sac is flask-shaped and obliquely placed and the vitellaria are postacetabular in distribution ; and from *C. macrotesticularis* which possesses deeply lobed testes, the genital pore more or less in level with the ventral sucker, and the intestinal caeca showing many undulations in the posttesticular region.

In the general shape of the body *C. oschmarini*, *C. indicus*, *C. odhnerensis* and *C. ocadiae* approach *C. sutlejensis* but there are many other differences. *C. sutlejensis* stands apart from *C. oschmarini* in which the testes are entire and oval, the ovary is rounded, and the cirrus sac is flask-shaped and obliquely placed; from *C. indicus* in which the testes are deeply lobed and the vesicula seminalis lies behind the cirrus sac; from *C. odhnerensis* in which the testes are small and irregularly lobed and the vesicula seminalis lies opposite to the crescent-shaped cirrus sac; and from *C. ocadiae* in which the testes are small and oval and the vesicula seminalis lies anterodorsally to the elongated and conical cirrus sac. *C. sutlejensis* stands very close to *C. yoshidai* in having the cirrus sac behind the vesicula seminalis but the position and the shape of the ovary and the commencement of the vitellaria are the characters which differentiate the two species; in *C. yoshidai*, the ovary is median and transversely elongated and the vitellaria commence behind the intestinal bifurcation.

Hosts : *Kachuga sylhetensis* (Jerdon)

Lissemys punctata punctata Bonnaterre

Location : Heart

Localities : Ropar and Sangrur (Punjab), Lucknow (U.P.)

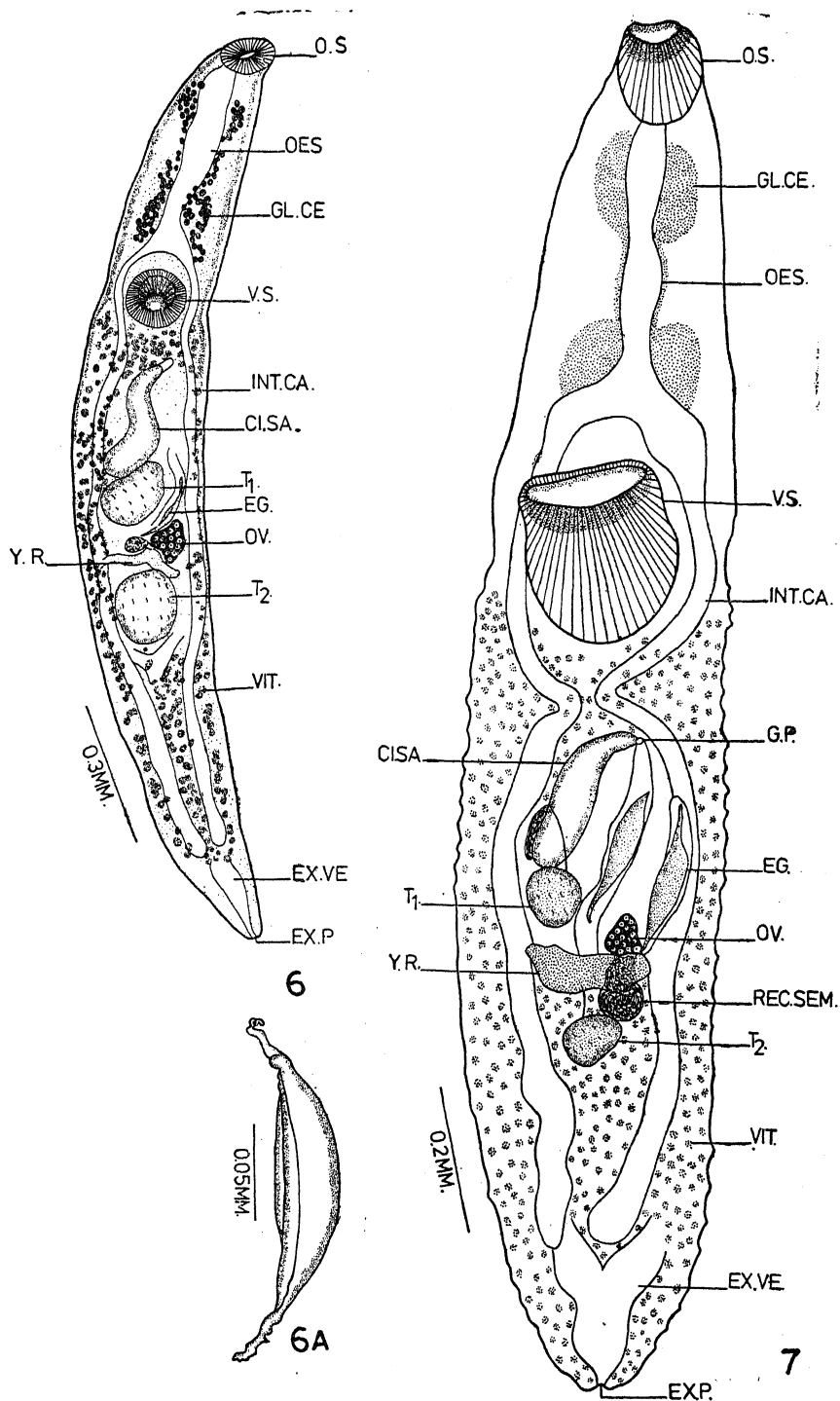
Coeuritrema sheilae Mehrotra, 1973 (figures 6, 6a)

The material consisted of eight specimens collected from the heart, blood and teased hepatic tissue of *Lissemys punctata punctata* Bonnaterre in Rudrapur (U.P.), Patiala and Sangrur (Punjab) and *Kachuga tectum tectum* (Gray) in Ropar (Punjab). The number of specimens in one host was never more than two. Of the flukes recovered, two were immature and one was distorted.

Description (five specimens measured): Body elongated, somewhat tapering towards extremities, 1.61–2.11 in length and 0.18–0.28 in maximum width across testicular region. Body surface smooth. Oral sucker 0.06–0.12 long by 0.05–0.09 wide. Ventral sucker just behind intestinal bifurcation, 0.11–0.13 × 0.09–0.16, larger than oral sucker. Oesophagus a wide tube, 0.29–0.41 long, surrounded by gland cells. Intestinal caeca slender, bending a little inwards just behind ventral sucker and again turning outwards and running parallel to body margins, converging behind posterior testis and continuing as straight tubes, terminating symmetrically 0.13–0.19 in front of posterior end of body.

Excretory system Y-shaped, pore terminal at rear extremity of body.

Gonads in middle third of body. Testes rounded or irregular, anterior testis 0.12–0.16 × 0.11–0.13, posterior testis 0.11–0.16 × 0.11–0.14. Vesicula seminalis externa (observed in two specimens only) small, opposite to basal portion of cirrus sac; the latter elongated, somewhat sinuous, placed more or less obliquely or longitudinally between ventral sucker and anterior testis, 0.24–0.28 long by 0.11–0.14 wide across its basal region, enclosing a small vesicula seminalis interna, pars prostatica and protrusible cirrus. Genital pore a little behind ventral sucker, sinistral, close to left intestinal caecum, may be inter or extracaecal (since inward bending of the intestinal caeca has been found to be variable, depending upon the flattened state of the fluke).



Figures 6, 6A, 7. See page 282 for caption.

Ovary intertesticular, sinistral, somewhat triangular, base of triangle being parallel to lateral margin of body and apex directed towards median line, $0.08-0.12 \times 0.08-0.13$. Receptaculum seminis median. Uterus containing a single egg (observed in one specimen only) with its shell forming polar prolongations. Egg 0.186×0.029 (including length of polar prolongations). Vitellaria beginning immediately behind ventral sucker and extending up to ends of intestinal caeca overlapping the latter and filling the entire posttesticular intercaecal space.

Remarks : In shape and disposition of the cirrus sac and also the position of the vesicula seminalis externa (i.e., opposite to the basal portion of the elongate cirrus that lies somewhat obliquely), *C. sheillae* shows its closest resemblance to *C. ocadiae* Takeuti 1942 and *C. odhnerensis* Mehra 1933, and differs from all the other known species of the genus. However, *C. ocadiae* and *C. odhnerensis* can also be differentiated from it because of the oral sucker being larger than the ventral and the vitellaria extending into the preacetabular zone in them, whereas in *C. sheillae* the oral sucker is smaller than the ventral and the vitellaria are restricted to the postacetabular region of the body.

Hosts : *Lissemys punctata punctata* Bonnaterre,
Kachuga tectum tectum (Gray)

Location : Heart, blood vessels, liver

Localities : Rudrapur (U.P.), Patiala, Sangrur and Ropar (Punjab)

Coeuritrema lyssimus Mehra, 1933 (figure 7)

Hosts : *Lissemys punctata punctata* Bonnaterre

Location : Heart

Locality : Rudrapur (U.P.)

Remarks : The present collection consisted of two specimens of *Coeuritrema lyssimus* Mehra 1933. Variations from the original description are : smaller gonads, the genital pore inner to the left intestinal caecum and the presence of two eggs in the uterus. According to Mehra (1933), the genital pore is external to the left intestinal caecum and the uterus contains only one egg at a time.

Rudrapur (U.P.) is a new locality record for this species.

In view of the observations on the species described by the authors and also of the descriptions of *C. odhnerensis* Mehra 1933 and *C. ocadiae* Takeuti 1942, a few generic characters of *Coeuritrema* as given by Yamaguti (1958, 1971) have been emended. The emended characters (italicised) are as follows :

Ventral sucker larger *or smaller than* or *equal to* oral sucker. Vesicular seminalis externa anterior, posterior or *opposite* to cirrus sac. Parasitic in blood vessels, *liver* or heart of freshwater chelonians.

KEY TO THE SPECIES OF THE GENUS *COEURITREMA* MEHRA, 1933

1. Body surface with conical tubercles or papillae.

... *C. lyssimus* Mehra, 1933

Body surface smooth

... 2

2. Cirrus sac somewhat oval, placed transversely to vertical axis of body. Vesicula seminalis externa in front of cirrus sac

... 3

Cirrus sac elongate, flask-shaped, placed obliquely. Vesicula seminalis externa behind cirrus sac or opposite to it

... 4

3. Ovary elliptical, median. Vitellaria commencing at a level behind intestinal bifurcation ... *C. yoshidai* (Ozaki 1939) Takeuti 1942.
Ovary elongated, sinistral. Vitellaria commencing at the level behind intestinal bifurcation ... *C. sutlejensis* Mehrotra, 1973
4. Vitellaria commencing behind ventral sucker ... *C. sheilae* Mehrotra, 1973
Vitellaria commencing in front of ventral sucker, i.e., at bifurcal level ... 5
Vitellaria extending throughout the body ... 7
5. Ventral sucker larger than the oral sucker. Intestinal caeca forming undulations in posttesticular region. Testes large, deeply lobed. Genital pore at level of ventral sucker ... *C. macrotesticularis* Rohde, Lee et Lim, 1968
Ventral sucker smaller than oral sucker. Intestinal caeca straight in posttesticular region. Testes relatively small, not deeply lobed. Genital pore quite behind ventral sucker ... 6
6. Ventral sucker close behind intestinal bifurcation, both intestinal caeca bending inwards behind it. Testes lobed ... *C. odhnerensis* Mehra 1973
Ventral sucker some distance behind intestinal bifurcation, only left intestinal caecum bending inwards behind it. Testes entire ... *C. ocadiae* Takeuti 1942
... *C. oschmarini* Belous 1963
Testes deeply lobed. Ovary elongated and lobed. ... *C. indicus* (Thapar 1933) Mehra 1934.
7. Testes oval, entire, ovary rounded. ... *C. oschmarini* Belous 1963
Testes deeply lobed. Ovary elongated and lobed. ... *C. indicus* (Thapar 1933) Mehra 1934.

References

- Belous E V 1963 Helminth fauna of *Amyda sinensis*; *Helminthologia* 4 79-88
- Dwivedi M P 1967 Contribution to the family Spirorchidae Stunkard, 1961 (Digenea: Trematoda); *Indian J. Helminthol.* 9 1-14
- Mehra H R 1933 New blood-flukes of the family Spirorchidae Stunkard, from Indian fresh-water tortoise with discussion on the systematic position of the genus *Coeuritrema* n.g and the relationships of families of blood flukes Part I; *Bull. Acad. Sci. U.P. Allahabad* 2 203-222
- Mehra H R 1934 New blood-flukes of the family Spirorchidae Stunkard, from Indian fresh water tortoises with discussion on the synonymy of certain genera and relationships of the families of blood-flukes. Part II; *Bull. Acad. Sci. U.P. Allahabad* 3 169-196
- Mehrotra V 1973 Digenea from some reptilian hosts in India; Parts I, II; *Proc. 60th Indian Sci. Cong.* Part IV 46-47
- Ozaki Y 1939 A new blood-fluke, *Hapalorhynchus yoshidai*; *Vol. Jubil. Pro Prof. Yoshida* 1 29-35
- Rohde K, Lee S K and Lim H W 1968 Ueber drei malayische Trematoden; *Ann. Parasit. Hum. Comp.* 43 33-34
- Srivastava H D 1960 Presidential address; 47th *Indian Sci. Cong.* Bombay
- Stunkard H W 1921 Notes on North American Blood flukes; *Am. Mus. Novit.* 12 1-5
- Takeuti E 1942 New blood flukes of the family Spirorchidae from Japanese fresh-water tortoise and marine turtles; *Jpn. J. Med. Sci. Pt. 6, Bacteriology and Parasit.* 2 161-174
- Thapar G S 1933 A new blood-fluke from an Indian tortoise, *Trionyx gangeticus*; *J. Helm.* 11 163-168
- Yamaguti S 1958 *Systema Helminthum. Vol. I. The digenetic trematodes of vertebrates* (Pt. I and II); (New York and London: Interscience Publishers)
- Yamaguti S 1971 *Synopsis of digenetic trematodes of vertebrates I*; (Japan: Keigaku Publishing Co.) pp. 1-1074

Figures 6, 6A and 7. 6. *Coeuritrema sheilae* Mehrotra 1973 (whole mount, ventral view); 6A. egg of the same. 7. *Coeuritrema lyssimus* Mehra, 1933 (whole mount, ventral view). (CL.SA., cirrus sac; EG, egg; EX.P., excretory pore; EX.VE, excretory vesicle; G.P., genital pore; GL.CE., gland cells; INT.CA., intestinal caeca; L.C.P., Lauer's canal pore; MT, metraterm; O.S., oral sucker; OES, oesophagus; OV, ovary; REC.SEM., receptaculum seminis; T₁, anterior testis; T₂, posterior testis; V.S. ventral sucker; VES.SEM.EXT., vesicula seminalis externa; VIT, vitellaria; Y.R. yolk reservoir).

Life history and behaviour of the cyst nematode, *Heterodera oryzaicola* Rao and Jayaprakash, 1978 in Rice (*Oryza sativa* L.)

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Abstract. The embryonic development of the cyst nematode, *Heterodera oryzaicola* and its emergence from egg masses was completed within 8 to 9 days. The emerged second stage juveniles were attracted to roots of rice within 24 hr and penetrated the roots within 24 hr. After penetration, the endoparasitic juveniles developed into males within 14 days and white females within 20 days. Orientation of females was equal towards hypocotyl (42%) and root tip (48%) while a few (10%) matured vertically in thin secondary roots or small rootlets. The sex-ratio between males and females was about 1 : 4. The virgin females secreted a strong male attractant and the males migrated towards the females in response to this stimulus and mated with the females. The eggs were laid in gelatinous matrix secreted by the females within 22 days and the females turned into brown cysts by 24 days. A single *H. oryzaicola* female laid on an average 198 eggs in an egg mass and retained 120 eggs within the body of the brown cyst. None of the females was left unmated and all of them laid a single egg mass each within 30 days. One life cycle was completed in 30 days and 12 generations occurred in a year.

Keywords. *Heterodera oryzaicola*; *Oryza sativa*; life history; behaviour.

1. Introduction

Severe leaf chlorosis, stunting and mortality of rice plants were observed in upland rice fields of Pattambi and its vicinity in Kerala State. Inoculation to rice cv. CRM 13-3241 under greenhouse condition proved that it was due to a new root infesting cyst nematode (Rao and Jayaprakash 1977). The nematode was subsequently described as *Heterodera oryzaicola* (Rao and Jayaprakash 1978). Information on the life history and behaviour of this new nematode was essential for adopting control measures in infested soil. Hence, the present investigation was taken up.

2. Materials and methods

2.1. Embryonic development

Freshly laid eggs in egg masses attached to the posterior end of mature white females of *H. oryzaicola* from roots of rice were removed and kept in hanging

drops of water on a microslide (Dasgupta and Raski 1968). The eggs were incubated at $28 \pm 1^\circ \text{C}$. Cell division, blastulation, development and eclosion of the juveniles were observed at intervals of 2 hr and recorded from 20 synchronous eggs of each egg mass.

2.2. *Post-embryonic development*

Plastic pots ($6 \times 6 \text{ cm}$) were each filled with 100 g of soil to which 40 ml water was added and one seed of rice was sown for germination. When sprouts were 10 days old, each was inoculated with 100 second stage juveniles of *H. oryzae*. At intervals of 2 days, roots of 4 sprouts were collected and examined for endo and semi-endoparasitic stages till brown cyst formation occurred in the roots. The juveniles and adults were recorded. The first appearance of any juvenile stage was considered as the result of its growth and moulting and accordingly, the duration of each stage was computed as the period between the first appearance of two successive stages.

Seedlings of rice were raised and inoculated with *H. oryzae* as above on the 1st day of every month for 12 months. Seedlings were sampled on any day after the 21st day. The number of cysts and white females per plant root system was recorded and they were kept in fresh rice root diffusates for hatching. The date of the first juvenile emergence was recorded and used as an indication of completion of a generation.

2.3. *Migration of infective juveniles towards rice roots*

Sprouts of rice were grown in petri dishes containing 1% agar media and when the sprouts were 10 days old, 100 freshly hatched second stage juveniles of *H. oryzae* were released at a distance of 10 mm from the nearest root. A mechanical barrier (by negative film strip or cover glass or any handy items can be used to make mechanical barrier) to prevent advancing of roots towards the site of juvenile release was made. The distance of 10 mm was divided into ten zones and the number of second stage juveniles present in the zones were recorded at 6 hr interval till 24 hr.

2.4. *Penetration*

In 48 plastic micro-pots ($2.5 \times 2.5 \text{ cm}$) 5 g soil was filled and one seed of rice was sown. Water was added for germination and when sprouts were 10 days old, each was inoculated with 100 second stage juvenile of *H. oryzae* in 4 replicates. Inoculated plants were equilibrated at $28 \pm 1^\circ \text{C}$ and kept under light. Every 2 hr after inoculation up to 24 hr, four sprouts were sampled for study of juvenile penetration into roots.

2.5. *Mating behaviour*

Seeds of rice were germinated individually in petri plates containing 1% agar media and when sprouts were 10 days old, each was inoculated with a single second stage juvenile of *H. oryzae*. When the mature females developed in the roots,

10 males were released from a distance of 10 mm and the agar media was divided into 10 zones of 1 mm diameter. The number of males present in the zones was recorded every 5 min after release.

2.6. Orientation

The orientation of white females or brown cysts in roots of rice from post-embryonic development study was recorded.

2.7. Fecundity

Sprouts of rice were grown as in post-embryonic development study and each was inoculated with 100 second stage juveniles of *H. oryzae*. Sprouts were sampled every 2 days from the 20th to the 30th day following inoculation for enumeration of endo and semi-endoparasitic stages and egg masses. Soil was processed for extraction of second stage juveniles (Whitehead and Hemming 1965).

3. Results and discussion

3.1. Embryonic development

Mature white females laying eggs in gelatinous egg mass were frequent. The eggs when laid were unicellular. Two-celled stage was observed in 4 hr after oviposition and multicellular stage during the next 44 hr. Juvenile differentiation occurred in 88 hr. Fully developed second stage juveniles, folded 3 or 4 times inside the egg shell, were observed in 187 hr (figure 1). Eclosion occurred in 12 to 24 hr. The entire developmental duration was found to be 8 to 9 days. The eggs in egg mass were asynchronous and the second stage juveniles hatched on completion of the development inside the egg shell, whereas the eggs retained in mature cysts were synchronous and even after completion of development inside the egg they remained dormant. Similarly, the embryonic development of *H. oryzae* in rice from Ivory coast was reported to have been completed within 6–10 days (Brizuela and Merny 1964).

3.2. Post-embryonic development

Juveniles of third stage (8) (table 1) first appeared in roots on the 6th day after penetration indicating that the second stage juveniles had established, developed and moulted (figure 2B). The third moult occurred on the 10th day in the male (3) and on the 16th day in female juveniles (3). Third stage cuticle with the coiled fourth stage juveniles of male (3) (figure 2C) were observed on the 10th day after inoculation. Fourth stage juveniles of female (3) (figure 2D, E) appeared on the 14th day, while the adult males (3) moved out of the root on the same and by the 22nd day, the females started laying eggs in gelatinous ovi sac secreted around the vulva (figure 2F, G). The colour of the females (2) turned light brown by 24th day and dark as it matured. Second stage juveniles emerged out of the egg masses by 24th to 31st day (table 2).

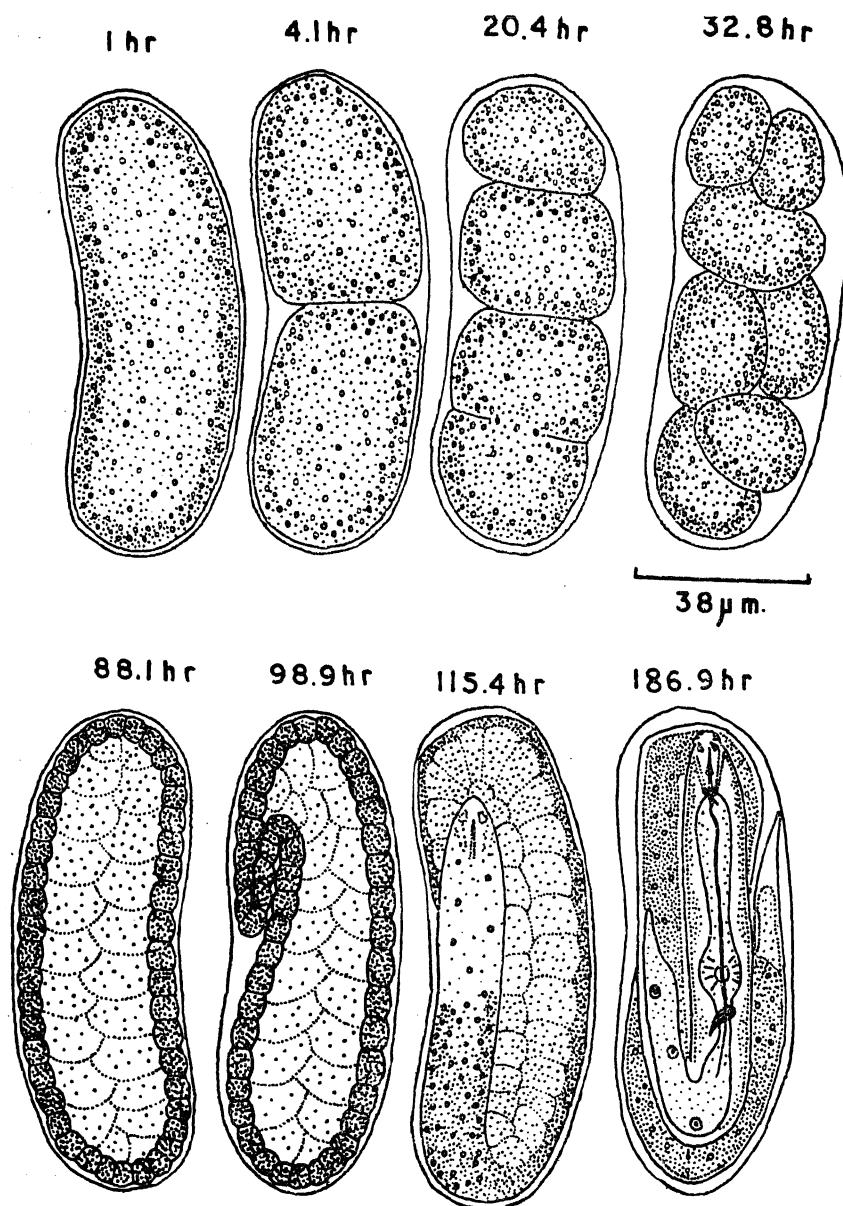


Figure 1. Embryonic development of *Heterodera oryzicola*.

The computed duration of development was 6 days for the second, 4 days for the third stage male and 8 days for the third stage female juveniles, while the fourth stage male and female juveniles require 4 and 8 days respectively to develop into adults. The sex ratio between males and females was 1 : 4 (table 1).

Table 1. Post-embryonic development of *H. oryzae* in roots of rice. Inoculum level 100 juveniles/seedling. (Average of 4 replicates).

Days after inoculation	Juveniles				Adults			Total
	II	III	IV		Male	Female		
			Female	Male		White with egg mass	Cyst with egg mass	
2	26	26	
4	31	31	
6	27	8	35	
8	11	20	31	
10	4	21	..	3	28	
12	..	24	..	6	30	
14	..	25	3	5	3	..	36	
16	..	16	10	2	6	..	34	
18	..	6	21	1	8	..	36	
20	14	..	8	10	32	
22	2	..	9	19 (5)	30	
24	4	26 (10)	32	
26	6	13 (13)	36	
28	5	6 (6)	32	
30	7	4 (4)	38	

Figures in parenthesis indicate egg masses.

Table 2. Number of generations of *H. oryzae* from March 1977 to February 1978 in rice under greenhouse conditions.

Date of inoculation	Date of observation	Number of cysts/plant root system	Date of first juvenile emergence	Generation
1- 3-1977	22- 3-1977	15.7	24- 3-1977	1st
1- 4-1977	24- 4-1977	14.1	25- 4-1977	2nd
1- 5-1977	22- 5-1977	24.4	29- 5-1977	3rd
1- 6-1977	26- 6-1977	28.3	24- 6-1977	4th
1- 7-1977	24- 7-1977	16.6	26- 7-1977	5th
1- 8-1977	22- 8-1977	13.7	25- 8-1977	6th
1- 9-1977	24- 9-1977	22.9	25- 9-1977	7th
1-10-1977	26-10-1977	21.2	28-10-1977	8th
1-11-1977	28-11-1977	20.4	29-11-1977	9th
1-12-1977	28-12-1977	12.8	30-12-1977	10th
1- 1-1978	26- 1-1978	15.9	31- 1-1978	11th
1- 2-1978	24- 2-1978	8.9	27- 2-1978	12th

One life cycle of *H. oryzae* was completed in 30 days from second stage juveniles of one generation to succeeding second stage. The life cycle was continuous and 12 generations were completed in a year under greenhouse conditions (table 2).

Males of *H. oryzae* developed in 14 days and females in 16 days (Brizuela and Merny 1964), while *H. vigni* took 13 days and 17 days respectively in roots of cowpea (Gupta and Edward 1973). *H. graminophila* in roots of barnyard grass (Birchfield 1970) and *H. zae* in roots of maize at 24–30 °C (Varma and Yadav 1975) took 20 days for female development, while *H. betulae* took 52 days at 28 °C (Riggs *et al* 1969). *H. avenae* completed one life cycle in 9–14 weeks in roots of wheat (Duggan 1961). Thus, it seems that the duration of post-embryonic development of *Heterodera* spp. varied with host plant and environmental temperature.

H. oryzae completed 12 generations in a year under greenhouse conditions though *H. trifolii* and *H. cajani* were reported to complete 9 generations in a year (Mulvey 1959; Koshy and Swarup 1971).

3.3. Migration of second stage juveniles towards rice roots

In 1% agar media the second stage juveniles migrated from the point of release towards the rice roots at 10 mm distance and above within 24 hr (figure 3). In 6 hr only 10 juveniles were present at 0.2 mm distance from the point of release, but by 12 hr 64% of the juveniles were present between 0.3 mm to 0.6 mm (2 to 15); by 18 hr 84% were present in between 0.5 mm to 0.8 mm (6 to 15) and by 24 hr 13 juveniles actually reached the roots, while 20 juveniles were close to the root system at 0.7 to 0.9 mm from the point of release. Hence, there was a steady and stable attraction of the juveniles towards the host roots. Similarly juveniles of *H. schachtii*, *H. avenae* and *G. rostochiensis* were also reported to accumulate around host roots (Baunake 1922; Wallace 1958; Kuhn 1959; Viglierchio 1961). Some bacteria from the rhizosphere of sugar-beet plants were reported to attract *H. schachtii* juveniles (Bergman and Van Duuren 1959). The present study on *H. oryzae* as well as those with *H. schachtii* and *H. oryzae* (Johnson and Viglierchio 1969; Reversat 1971) confirmed that attraction occurs in sterile condition also and hence it may be suggested that the stimulus for attraction emanated from the host roots.

3.4. Penetration

At constant temperature of $28 \pm 1^\circ \text{C}$, the second stage juveniles of *H. oryzae* penetrated into the roots of rice (table 3). Within 2 hr following inoculation, a few second stage juveniles (2) commenced penetrating into the epidermis and the maximum number of juveniles penetrated by 6–10 hr (20–29). With the advance in time, the juveniles reached the cortex in large numbers (18–35). Within 18 hr a few juveniles (2) had penetrated the endodermis and pericycle, but by 24 hr most of the juveniles reached to stele (27) (figure 2A). *H. glycines* and *H. vigni* also penetrated within 24 hr (Endo 1964; Gupta and Edward 1973), while *H. zae* penetrated within 48 hr to 72 hr (Varma and Yadav 1975).

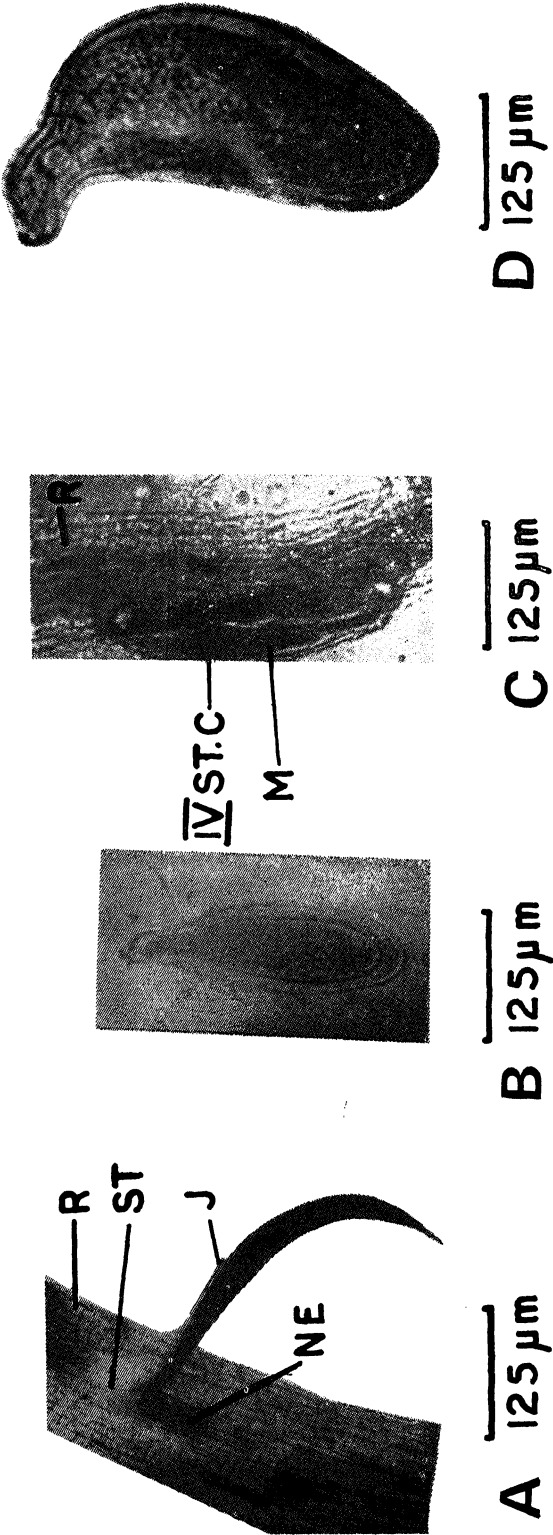
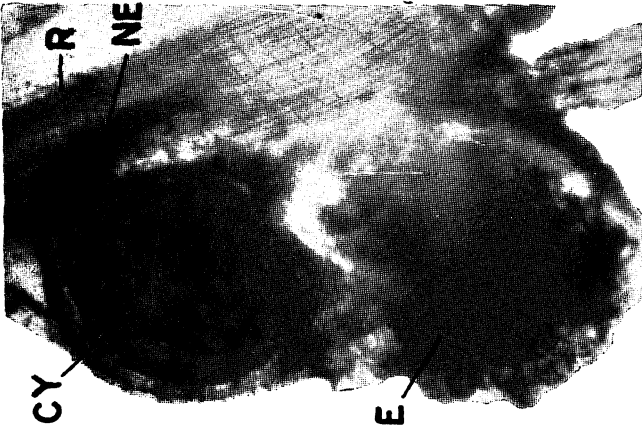
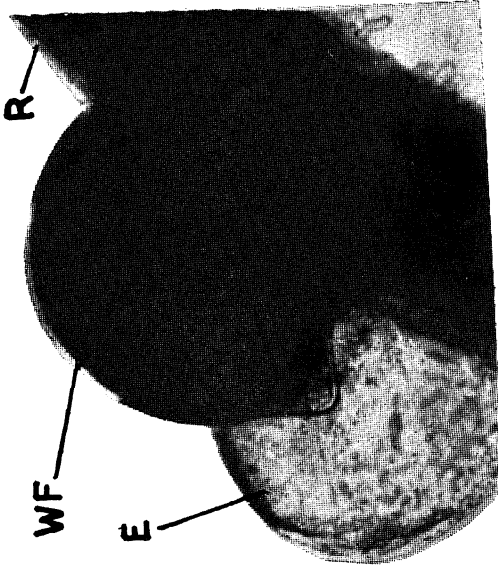


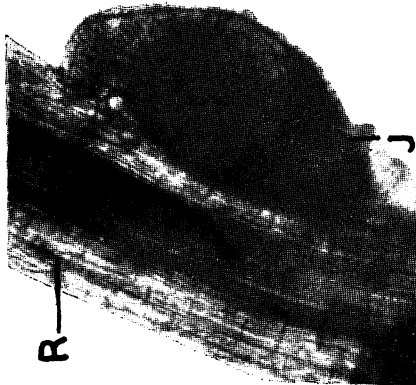
Figure 2. Post-embryonic development of *Heterodera oryzaicola* in rice roots. A. Second stage juvenile (J) penetrating rice root (R) and feeding on the stele (ST) resulting in necrosis (NE); B. Third stage juvenile; C. Fourth stage male (M) and the fourth stage male cuticle (IV ST. C); D. Fourth stage female juvenile.



G 200 μm



F 150 μm



E 145 μm

Figure 2E-G. E. Fourth stage female juvenile (J) feeding on root (R) with the head embedded into the stele; F. Adult virgin white female (WF) with head embedded into the root (R) with an egg mass secreted around the vulva plain without eggs; G. Cyst (CY) with head embedded into the root (R) causing necrosis (NE) and egg mass filled with eggs.

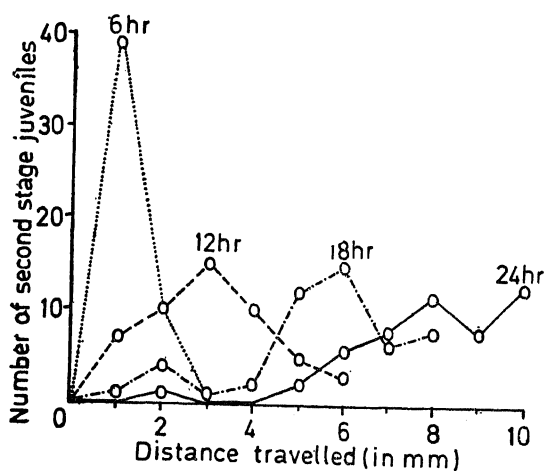


Figure 3. Migration of second stage juveniles towards rice roots.

Table 3. Penetration by *H. oryzae* juveniles into roots of rice. Age of plant at inoculation = 10 days. Inoculum level = 100 second stage juveniles/seedling. Temperature = $28 \pm 1^\circ\text{C}$ (Average of 4 replicates).

Time in hr	Number of second stage juveniles penetrated			
	Epidermis	Cortex	Endodermis and Pericycle	Total
2	2	2
4	10	10
6	20	4	..	24
8	28	8	..	36
10	29	5	..	34
12	10	18	..	28
14	7	25	..	32
16	2	31	..	33
18	..	35	2	37
20	1	20	10	31
22	..	10	22	32
24	..	2	27	29

3.5. Mating behaviour

In 1% agar media, the males migrated towards the virgin females of *H. oryzae* from the point of release at a distance of 10 mm within 25 min (figure 4). Most of the males reached 3 to 6 mm distance from the point of release in 10 min (1-4), by 15 min in 6 to 9 mm (1-4) and by 20 min in 8 to 10 mm (1-7). After 25 min, 8 males had actually reached the females indicating the existence of a

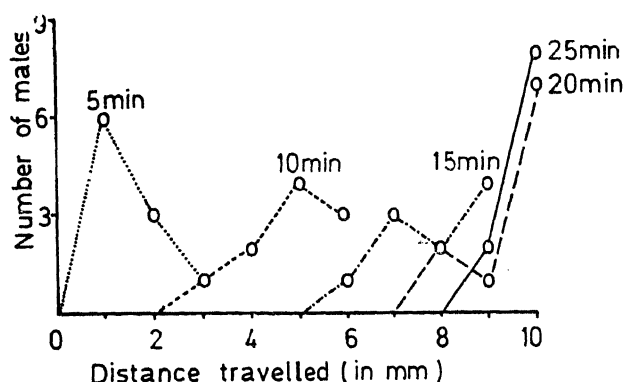


Figure 4. Migration of males towards virgin females of *H. oryzicola*.

strong male attractant force emanating from virgin females. Mating lasted for an hour and more than one male took part in it. The gelatinous egg sac present around the vulva was not observed to interfere in mating process. After the mating the males moved away and did not respond further to the male attractants. None of the females subjected to experimentation were left unmated. The secretion of male attractants and migration of males towards female were so far reported in 10 species of *Heterodera* and *Globodera* and most females secreted more than one attractant and most males responded to more than one (Green 1966, 1967; Fox 1967; Greet *et al* 1968; Green and Plumb 1970). The male attractants of *G. rostochiensis* and *H. schachtii* were also reported to spread or diffuse in solution, volatilize in air and accumulate in agar blocks (Green 1967; Greet *et al* 1968). In a 3 mm thick agar block it took more than 15 min at 20° C to diffuse sufficiently to be perceptible to males 5 mm from the females of *G. rostochiensis* and *H. schachtii* (Green 1966).

3.6. Orientation

In this test, an average of 12.5 females of *H. oryzicola* in the roots were found oriented towards hypocotyl, 14.5 towards the root tip and 3 matured perpendicular to the long axis of root in secondary roots or small rootlets with their head ends embedded inside the root (table 4). Almost all the females ruptured the cortex to be exposed outside and the egg sacs remained completely outside the root. Orientation of females of *H. schachtii* towards hypocotyl and root tip was equal and some juveniles matured at the root surface of sugar-beet to a varying degree ranging from completely endoparasitic to nearly completely ectoparasitic. The juveniles maturing at the root surfaces invariably developed into males (Steele 1977).

3.7. Fecundity

The mature white females and brown cysts with or without egg sac formed in root system of 6 rice plants during the 30 days following inoculation with 100 second stage juveniles of *H. oryzicola* varied from 4 to 31 (table 5). The cumulative

Table 4. Orientation of *H. oryzae* females in roots of rice. Age of seedling at inoculation = 10 days. Inoculum level = 100 second stage juveniles/seedling (Average of 6 replicates).

Mean number of cysts/white females oriented towards		
Hypocotyl	Root tip	Vertically in secondary roots
12.5	14.5	3.0

Table 5. Oviposition of *H. oryzae* in rice. Inoculum level = 100 second stage juveniles/seedling.

Inoculation No.	Days after inoculation									
	22		24		26		28		30	
	W/C	E	W/C	E	W/C	E	W/C	E	W/C	E
1	12	..	20	8 (8)	21	15 (120)	29	21 (1535)	28	28 (1085)
2	15	..	15	4 (10)	23	17 (245)	26	22 (1815)	27	27 (957)
3	4	..	16	8	20	7 (88)	26	26 (2318)	21	21 (1578)
4	12	..	20	12 (15)	25	18 (214)	25	23 (2275)	22	22 (3105)
5	11	..	18	9	26	12 (136)	21	20 (3408)	28	26 (1789)
6	8	..	21	5	22	19 (286)	27	22 (2557)	31	31 (2035)
Total	62	..	110	46 (33)	137	88 (1089)	154	134 (13908)	157	155 (10549)
Average No. of eggs/female	0	..	38	27	112	98	115	195	120	198
Average No. of egg masses/day	0	..	0.084		0.044		0.046		0.024	

W = White females or C = Cysts; E = Egg masses.
 Figures in parenthesis are hatched second stage juveniles.

number of egg masses (hatched and unhatched) laid per female increased from 33 on 24th day to 155 on the 30th day indicating that all the egg masses were formed within this period. The average daily oviposition rate was 0.084 on 24th day and after which there was a gradual decline to 0.024 on the 30th day. At 30 days, the average number of eggs laid in egg mass was 198 and retained in cysts was 120 per single female. The cysts of *H. avenae* were found to retain over 600 eggs (Anderson 1961) and *H. schachtii* retained from 10 to over 600 with an average of 286 from 500 Utah specimens. The eggs laid in egg sac of *H. schachtii*, *H. glycines*, *H. trifolii*, *H. cruciferae* and *H. carotae* were reported to be as many as 200, in *H. fici* about 100 and in *H. goettingiana*, *H. avenae* and some large specimens of *H. galeopsidis* laid quite a few eggs only (Thorne 1961).

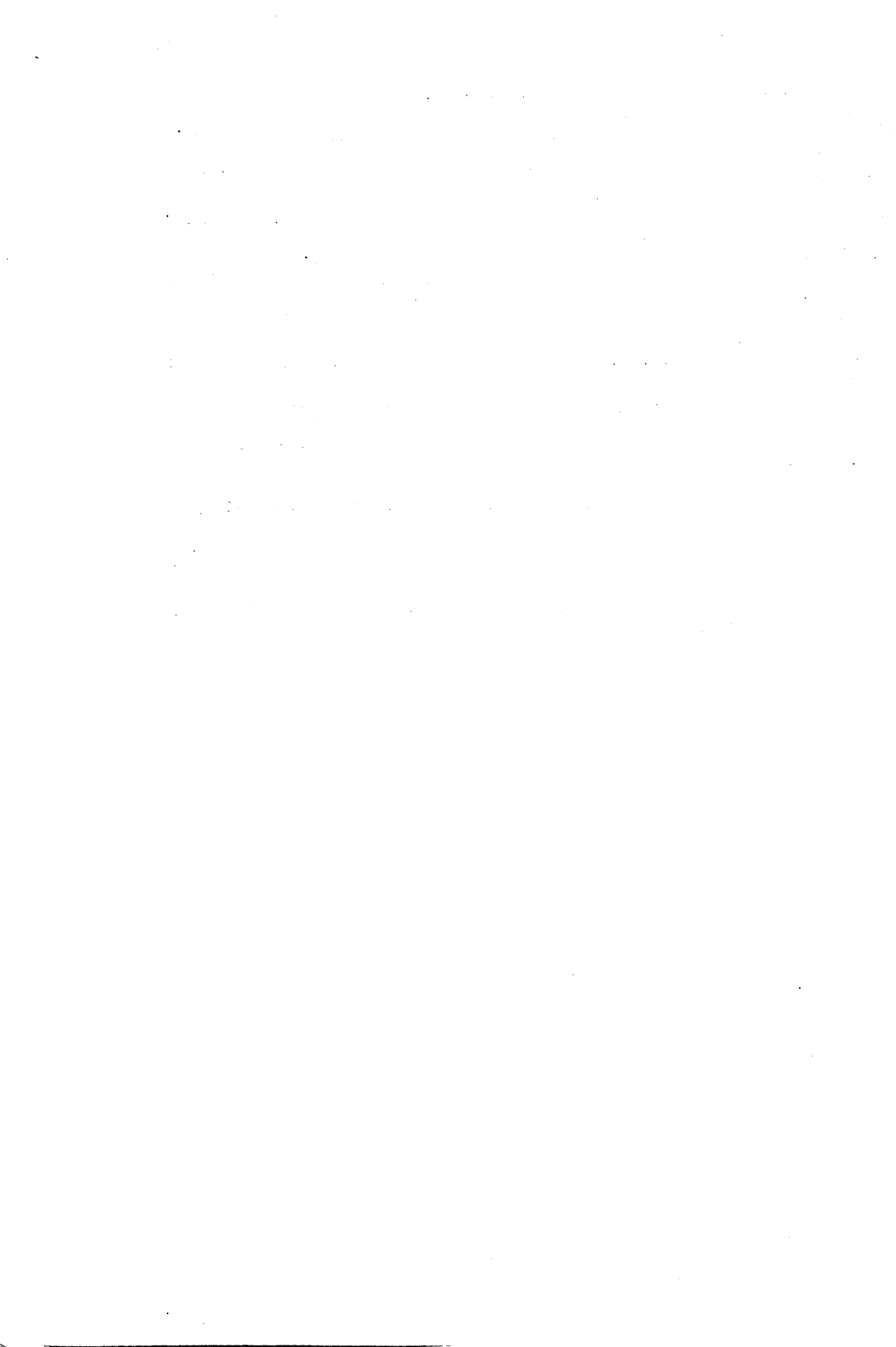
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References

- Andersen S 1961 Resistens mod havreal *Heterodera avenae*; *Medd. Vet. Hisk. Afd. landbr. Plkult. Nr.* 68 179
- Baunacke N 1922 Untersuchungen zur Biologie und Bekämpfung des Rubennematoden, *Heterodera schachtii* Schmidt; *Arb. Biol. Reichanst. Landw. Forstw.* 11 185-288
- Bergman B H H and Van Duuren A J 1959 Sugar-beet eelworm and its control. VI. The influence of host plant roots and their secretion products on the orientation of *Heterodera schachtii* larvae *in vitro*; *Meded. Inst. Suikerbeit* 29 1-24
- Birchfield W 1970 The biology of a new cyst nematode on grasses (Abstract); *Phytopathology* 60 1284-1285
- Brizuela R B and Merny G 1964 Biologie d'*Heterodera oryzae* Luc and Berden, 1961 I. Cycle du parasite et reactions histologiques de l'hôte; *Rev. Path. Veg. Entomol. Agric.* 43 43-53
- Dasgupta D R and Raski D J 1968 The biology of *Rotylenchulus parvus*; *Nematologica* 14 429-440
- Duggan J J 1961 Seasonal variations in the activity of cereal root eelworm (*Heterodera major* O. Schmidt, 1930); *Sci. Proc. R. Dublin Soc. Ser. B1* 21-24
- Endo B Y 1964 Penetration and development of *Heterodera glycines* in soybean roots and related anatomical changes; *Phytopathology* 54 79-88
- Fox J A 1967 Reproductive isolation in the genus *Heterodera*; *Nematologica* 13 143-144
- Green C D 1966 Orientation of male *Heterodera rostochiensis* Woll. and *H. schachtii* Schm. to their females; *Ann. Appl. Biol.* 58 327-339
- Green C D 1967 The attraction of male cyst nematodes by their females; *Nematologica* 13 172-173
- Green C D and Plumb S C 1970 The interrelationships of some *Heterodera* spp. indicated by the specificity of the male attractants emitted by their females; *Nematologica* 16 39-46
- Greet D N, Green C D and Poulton M E 1968 Extraction, standardization and assessment of the volatility of the sex attractants of *Heterodera rostochiensis* Woll. and *H. schachtii* Schm.; *Ann. Appl. Biol.* 61 511-519
- Gupta P and Edward J C 1973 Studies on the biology of *Heterodera vigni* (Heteroderidae: Nematoda) I. Life cycle; *Indian J. Nematol.* 3 99-108
- Johnson R N and Viglierchio D R 1961 The accumulation of plant parasitic nematode larvae around carbon dioxide and oxygen; *Proc. Helminthol. Soc. Wash.* 28 171-174
- Johnson R N and Viglierchio D R 1969 Sugar beet nematode (*Heterodera schachtii*) reared on axenic *Beta vulgaris* root explants I. Selected environmental factors affecting penetration; *Nematologica* 15 129-143

- Koshy P K and Swarup G 1971 On the number of generations of *Heterodera cajani*, the pigeon-pea cyst nematode in a year; *Indian J. Nematol.* 1 88-90
- Kuhn H 1959 Zum problem der wirksfindung phytopathogene nematodan; *Nematologica* 4 165-171
- Mulvey R H 1959 Susceptibilities of plants to clover cyst nematode, *Heterodera trifolii* and the period required to complete a life cycle; *Nematologica* 4 132-135
- Rao Y S and Jayaprakash A 1977 Leaf chlorosis in rice due to root infestation by a new cyst nematode; *Int. Rice Res. News Lett.* 2 5
- Rao Y S and Jayaprakash A 1978 *Heterodera oryzicola* n.sp. (Nematoda : Heteroderidae) a cyst nematode on rice (*Oryza sativa* L.) from Kerala State, India; *Nematologica* 24 341-346
- Reversat G 1971 *Contribution al'etude dela Biologie d'un nematode phytoparasite, Heterodera oryzae*; Ph.D. Thesis (Univ. Claude-Bernard de Lyon)
- Riggs R D, Hirschmann H and Hamblen M L 1969 Life cycle, host range and reproduction of *Heterodera betulae*; *J. Nematol.* 1 180-183
- Steele A E 1971 Orientation and development of *Heterodera schachtii* larvae on tomato and sugar-beet roots; *J. Nematol.* 3 424-425
- Thorne G 1961 *Principles of Nematology* (New York, Toronto and London : McGraw-Hill)
- Varma A C and Yadav B S 1975 Life history of *Heterodera zeae* on maize under Udaipur conditions; *Indian J. Mycol. Pl. Path.* 5 19
- Viglierchio D R 1961 Attraction of parasitic nematodes by plant root emanations; *Phytopathology* 51 136-143
- Wallace H R 1958 Observations on the emergence from cysts and the orientation of larvae of three species of the genus *Heterodera* in the presence of host plant roots; *Nematologica* 3 236-243
- Whitehead A G and Hemming J R 1965 A comparison of some quantitative methods of extracting small vermiform nematodes from soil; *Ann. Appl. Biol.* 55 25-38



Sediment-ostracode relationship in the Bimili backwater and the Balacheruvu tidal stream

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Abstract. Based on the collections of benthic ostracodes during January-December 1977 from two selected marginal water bodies, namely Bimili backwater and Balacheruvu tidal stream on the east coast of India, the quantitative variations in the ostracode fauna have been studied in relation to the sedimentological characteristics like sand, silt and clay and organic matter content.

Keywords. Marginal water bodies ; sedimentological characteristics ; organic matter in sediment ; ostracode assemblages.

1. Introduction

Studies on sediment-ostracode relationship are rare and whatever is available are mainly concerned with the distribution of dead fauna. Moreover, information on the distribution, sedimentological and ecological relationship of living benthic ostracodes has been published either in taxonomic papers or in publications principally concerned with the ecology of other groups. The studies of Remane (1933), Klie (1936), Elofson (1941), Smidt (1951), Wieser (1959, 1960), Kornicker (1964), Kornicker and Wise (1960), Puri *et al* (1964), McIntyre (1964), Engel and Swain (1967), Williams (1969), King and Kornicker (1970), Joy and Clark (1977) and Athersuch (1979) have shown that the nature of the substratum and organic matter content play a vital role in controlling the biota in the habitat.

Malkin (1954) and Swain (1955) did not find any pronounced correlation between the distribution of ostracodes and character of the substratum. Kornicker (1958) found that the correlation was disappointing in the Bimili area, Great Bahama Bank, while Benson (1959) found that sediment had a marked influence on some of biofacies in Estero de Punta Banda.

In the present investigation an attempt has been made to establish a possible relationship between the ostracode fauna and the sediments in two selected bodies of water, Bimili backwater and Balacheruvu tidal stream.

2. Areas of investigation

Bimili backwater : The area covered is an extensive shallow backwater about 4.5 sq. km towards the north of Bheemunipatnam (Long. 83° 28' E ; Lat. 17°

54' N). Three nearly equidistant stations (I to III) are located for collection of samples (figure 1).

Balacheruvu tidal stream: This meandering stream opens into Bay of Bengal 15 km (by coast line) south of Visakhapatnam (Long. 83° 15' E; Lat. 17° 39' N). Three stations (I to III) are located in the course of the stream for the collection of samples (figure 2).

3. Material and methods

Collections were made at monthly intervals for one year (January–December 1977) at six fixed stations, three in the Bimili backwater and three in the Balacheruvu tidal stream. For quantification of ostracodes, collections were made using a device developed by Phleger (1960) and the density of ostracode fauna was expressed as number per 10 cm².

To study sediment composition and its organic matter, sediment was collected by pushing a PVC corer of 4.5 cm diameter. Sand, silt and clay fractions in the sediment were estimated by the pipette method of Krumbein and Pettijohn (1938). Organic matter was estimated by the method of Gaudette *et al* (1974).

4. Results

Seasonal variations in fauna in relation to sedimentological parameters are shown in figures 3 and 4. In the Bimili backwater, the organic matter content ranged from 0.32 to 4.12%. In general, higher values were recorded in July which marks the end of hot weather season and the establishment of the south-west monsoon season when drainage from the land was high. In addition, the contribution of organic matter by the decaying algae which grows densely on the western margin of the backwater is significantly high.

In the Balacheruvu stream, the organic matter content ranged from 0.34 to 3.56%. Higher values of organic matter were observed at station II compared to the values at stations I and III.

Sediment analyses show that sand was dominant over the silt and clay fractions at all the stations in the Bimili backwater and the Balacheruvu stream. Hence sediments of Bimili and Balacheruvu may be categorised as sandy areas following the categorisation of Folk (1968).

At station I in the Bimili backwater ostracodes were present in greater numbers from March through September than during the remainder of the year. Except for a peak in May, the abundance of ostracodes at station II did not vary markedly during the year. At station III ostracodes were encountered in considerable numbers in January, and from May to July.

In the Balacheruvu tidal stream ostracodes were encountered in considerable numbers in January, February and December collections. At station II, ostracodes were present from August to December in higher numbers compared to other months. Except for a peak number in January, the abundance of ostracodes at station III did not vary markedly during the year.

At stations I and II of Bimili and Balacheruvu the maximum in the seasonal abundance of live ostracodes coincided with the highest organic matter content. Slight deviation from this trend was seen at station III in both areas.

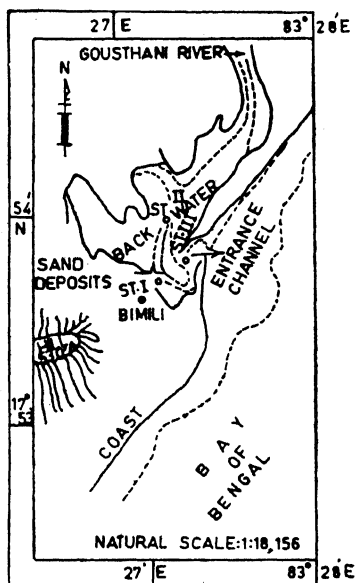


Figure 1. Location map of Bimili backwater.

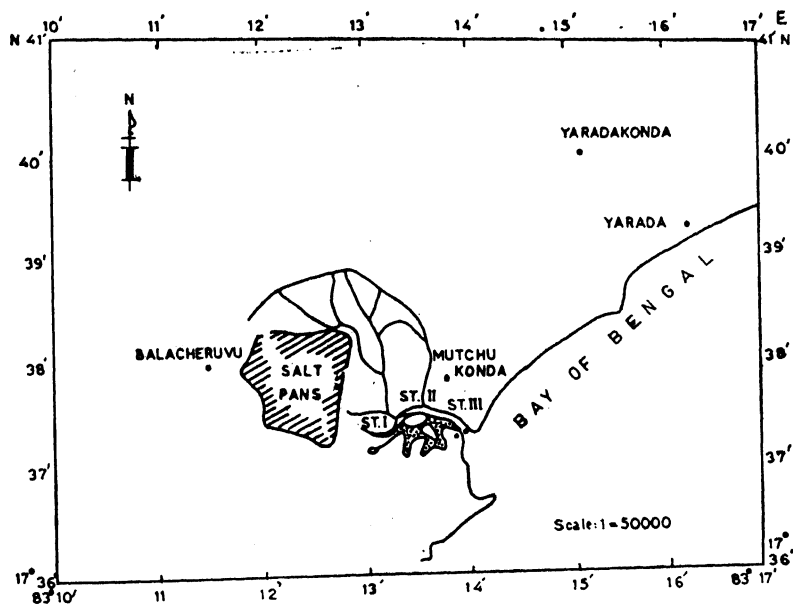


Figure 2. Location map of Balacheruvu tidal stream.

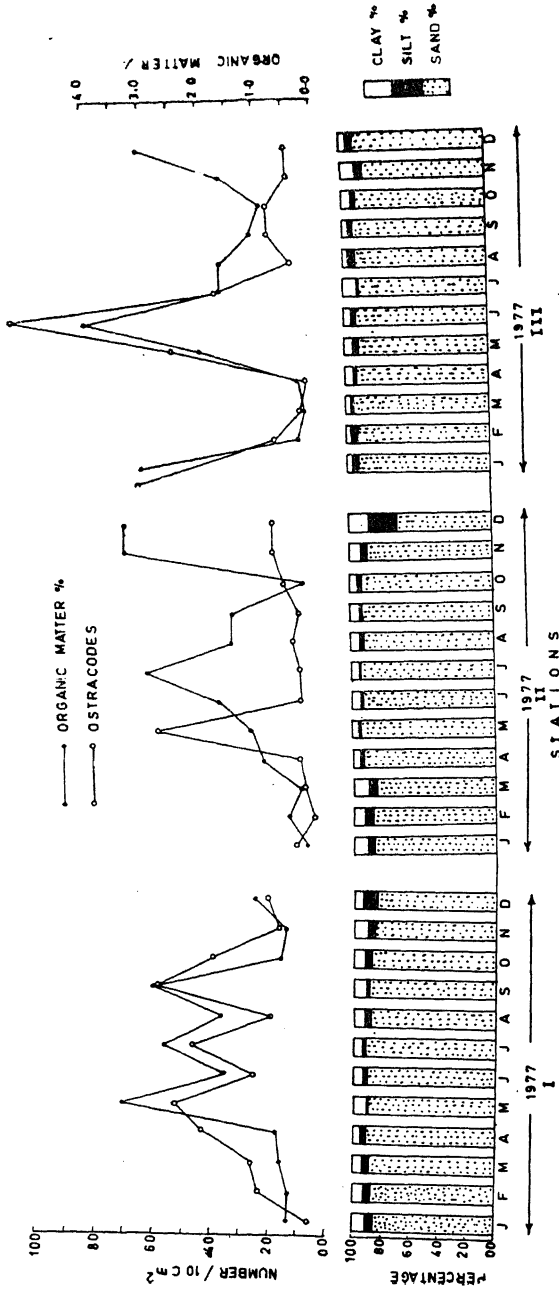


Figure 3. Monthly variations in organic matter, sand, silt and clay percentages in the sediments and ostracode fauna in the Bimili backwater.

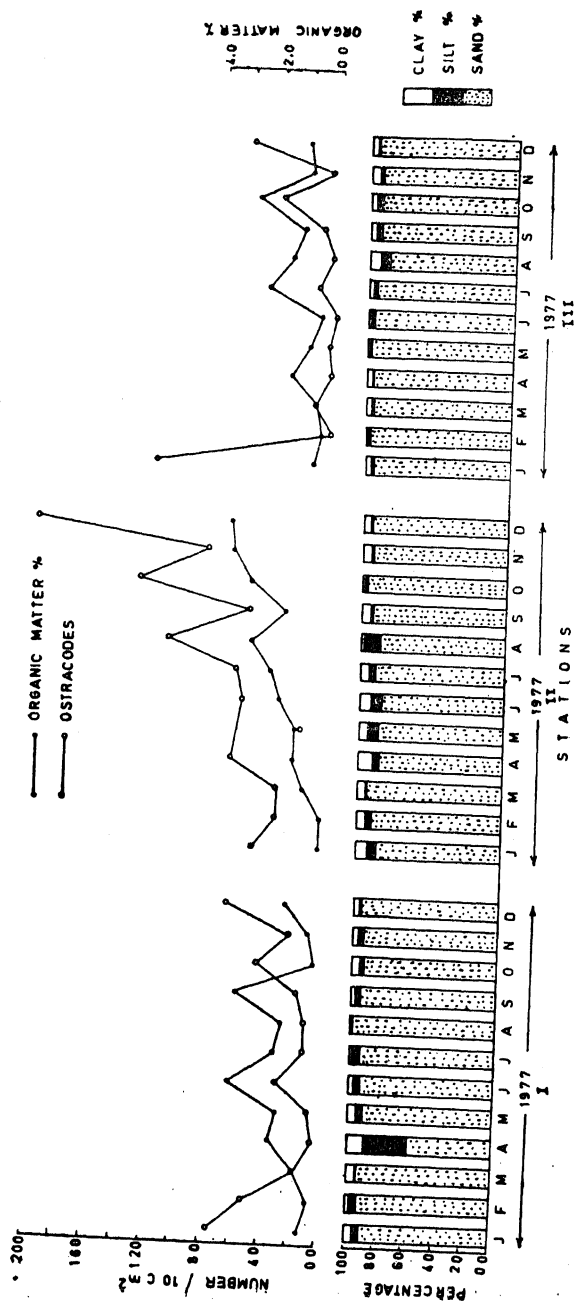


Figure 4. Monthly variations in organic matter, sand, silt and clay percentages in the sediments and ostracode fauna in the Balacheruvu tidal stream.

The sand, silt and clay fractions at the six stations during different months, when viewed in the background of total numbers of ostracodes, clearly indicate that ostracode abundance increased as the sand and clay content increased and silt content decreased. At station I in the Balacheruvu stream, the sand content was below 60% and silt above 10% in April and a fall in the ostracode numbers coincides.

Relatively higher numbers of ostracodes were encountered at station III of Bimili and station II of Balacheruvu, compared to the other stations. It is interesting to note that sediments at the above stations hold a higher sand and clay fraction and relatively high percentage of organic matter content.

5. Discussion

Throughout the survey conducted in Balacheruvu and Bimili backwater, samples contained faecal pellets in large quantities which the ostracodes seem to nibble indicating that the pellets form a sizable source of food. The fact that faecal pellets serve as the food source for the ostracode fauna is well established (King and Kornicker 1970).

The ostracode abundance in the areas of study increased with the availability of organic matter. The ostracode abundance varying with availability of food was observed by Swain (1955), Engel and Swain (1967) and Joy and Clark (1977).

A close examination of the pattern of distribution of ostracodes in relation to the sediment composition reveals that ostracodes prefer areas high in sand and clay fraction rather than silty areas. Thus Balacheruvu and Bimili sustain ostracodes in considerable numbers. This observation agrees with those made elsewhere in similar localities by Klie (1936), Elofson (1941), Smidt (1951), Benson (1959), Wieser (1959, 1960), McIntyre (1964) and Williams (1969).

High density of ostracodes observed in the shallow backwater and the tida stream is due to the high rate of photosynthesis of diatoms in the sediments. This observation agrees with those made elsewhere in similar localities by Hagermann (1967).

The stability structure of the sediment exerts a strong influence on the marine ostracodes in the selection of a suitable substratum. While the smooth-shelled forms prefer fine-grained muds, the rough and more ornate ostracodes prefer coarse or calcareous sediments. Such terms like endopelose (silt and clay burrowers), epipelose (silt and clay wanderers) and epipsammon (sand surface crawlers) have been suggested by Remane (1933) and Elofson (1941) for ostracode assemblages typical of certain bottom sediments which emphasize the control of the substrate over the character of associated assemblages.

In the present study smooth-shelled forms like *Phlyctenophora* occurred in sand-dominated areas but not in muddy areas. Forms such as *Tanella*, *Loxoconcha*, *Paijenborchellina* and *Atjehella* which are sculptured and heavily ornamented were encountered in considerable abundance in the sandy areas. *Palmenella* which is the characteristic genus of station III of Bimili backwater is known to be epipelitic (Remane 1938).

The foregoing account suggests that substratum plays a major role in the distribution of ostracodes both qualitatively and quantitatively. Regions of sandy sediments containing high percentage of organic matter content were more densely populated.

Acknowledgements

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References

- Athersuch J 1979 The ecology and distribution of the ostracodes of Cyprus ; *J. Nat. Hist.* **13** 135-160
- Benson R H 1959 Ecology of recent ostracodes of the Todos Santos Bay region, Baja California, Mexico, *Univ. Kansas. Paleont. Contrib. Arthropoda*, art p. 1-80
- Elofson O 1941 Zur kenntnis der mariner Ostracoden Schwedens, mit besonderer Berücksichtigung des Skagerracks ; *Uppsala Univ. Zool. Bidr.* **19** 215-534
- Engel P L and Swain F M 1967 Environmental relationships of Recent Ostracoda in Mesquite, Aransas and Copano Bays, Texas Gulf Coast ; *Trans. Gulf Coast. Assoc. Geol. Soc.* **17** 408-427
- Folk R L 1968 *Petrology of sedimentary rocks* (Texas : Hemphill's Austin) p. 170
- Gaudette H E, Wilson R F, Lois Toner and David W Folger 1974 An inexpensive titration method for the determination of organic carbon in Recent sediments ; *J. Sed. Petrol.* **44** 249-253
- Hagermann L 1967 Ostracodes of the Tvärminne area, Gulf of Finland ; *Commentat. Biol.* **30** 1-12
- Joy J A and Clark L D 1977 The distribution, ecology and systematics of the benthic Ostracoda of Central Arctic Ocean ; *Micropaleontology* **23** 129-154
- King C E and Kornicker L S 1970 Ostracoda in Texas Bays and Lagoons : An ecologic study ; *Smith. Contr. Zool.* **24** 92
- Klie W 1936 Ostracoden der Familie Cytheridae aus sand und Schell von Helgoland ; *Kieler Meeresforsch* **1** 49-72
- Kornicker L S 1958 Ecology and taxonomy of Recent marine ostracodes in the Bimini area, Great Bahama Bank, Texas ; *Texas Univ. Inst. Mar. Sci. Publ.* **5** 194-300
- Kornicker L S 1964 A seasonal study of living Ostracoda in Texas Bay (Redfish Bay) adjoining the Gulf of Mexico ; *Publ. Staz. Zool. Napoli* **33** Suppl 45-60
- Kornicker L S and Wise C D 1960 Some environmental boundaries of a marine ostracode ; *Micropaleontology* **6** 393-398
- Krumbein W C and Pettijohn F J 1938 *Manual of sedimentary petrography* (New York : Appleton-Century Crofts Inc.) p. 549
- Malkin D S 1954 Biostratigraphic study of Miocene Ostracoda of New Jersey, Maryland and Virginia ; *J. Palaeontol.* **27** 761-799
- McIntyre A D 1964 Meiobenthos of sublittoral muds ; *J. Mar. Biol. Ass. U.K.* **44** 665-674
- Phleger F B 1960 *Ecology and distribution of Recent Foraminifera* (Baltimore : The John's Hopkins Press) p. 297
- Puri H S, Bonaduce G and Malloy J 1964 Ecology of the Gulf of Naples ; *Publ. Staz. Zool. Napoli* **33** Suppl. 87-199
- Remane A 1933 Verteilung und organisation der benthonischen Mikrofauna der Kieler Bucht ; *Wiss. Meeresunters. Kiel.* **21** 161-221
- Smidt E L B 1951 Animal production in the Danish Wadden sea ; *Medd. Komm. Dann. Fiskeri-og. Havunders* **11** p. 151
- Swain F M 1955 Ostracodes of San Antonio Bay, Texas ; *J. Paleontol.* **29** 561-646
- Wieser W 1959 The effect of grain size on the distribution of small invertebrates inhabiting the beaches of the Puget Sound ; *Limnol. Oceanogr.* **4** 181-194
- Wieser W 1960 Benthic studies in Buzzards Bay II. The meiofauna ; *Limnol. Oceanogr.* **5** 121-137
- Williams R 1969 Ecology of the ostracoda from selected marine intertidal localities on the coast of Anglesey. In *Taxonomy, morphology and ecology of Recent Ostracoda* (ed.) J Neal (Edinburgh ; Oliver and Boyd) 229-327



Effect of DDT on brain neurosecretory cells of adult *Poekilocerus pictus* (Orthoptera : Acrididae)

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Abstract. Neurosecretory cells occur in groups, medially, dorsally, dorsolaterally, laterally and midventrally, in the protocerebrum and tritocerebrum of adult *Poekilocerus pictus*. Mid-brain is devoid of such cells. On the basis of staining reactions the NS cells have been differentiated into A and B types. The median group consists of about 50-55 A and 30 B cells lying on either side of the mid-line in the parsintercerebralis. The other parts of the protocerebrum and tritocerebrum are filled with only B cells. Scant NSM is found in the NS cells of freshly moulted adult. Synthetic activity increases with age and after about 5 or 6 days the cells contain deep staining secretory vesicles.

Treatment of 1-6 day old *P. pictus* with DDT for different periods showed that short incubation of 24 hr triggers the synthetic activity of NS cells, but prolonged incubation of 72 hr leads to a total depletion of NSM and to disruptive changes, like undulation of cell wall, cell shrinkage and ultimate cellular disruption.

Keywords. *Poekilocerus pictus* ; effect of DDT ; neurosecretory cells.

1. Introduction

Extensive literature is available on the morphology and histology of the neurosecretory system, but few workers have studied the changes in these cells induced under chemical stress (Matsuzawa 1964 ; Masner *et al* 1970 ; Ghosh *et al* 1968 ; Nanda 1970 ; 1973, 1974 ; Voitkevitch and Leonova 1964).

While studying the effect of tranquillizers at the level of brain nucleoprotein in *Periplaneta americana*, Ghosh *et al* (1968) reported a patchy condition of cytoplasm, vacuolation in cell perikarya and undulation of cellular membrane. Nanda (1973) reported various grades of depletion such as marginal depletion and accumulation of neurosecretory material in the neurosecretory cells of *P. americana*.

In insects chemical insecticides are known to interfere with many physiological and morphological functions. Ramade (1967) observed reduction of neurosecretory material in the brain neurosecretory cells of *Musca domestica* treated with BHC. Prasad and Srivastava (1980) also studied the effect of BHC on neurosecretory cells of *P. pictus*. Such studies in insects are relatively few and not

much is known about changes in cytoarchitecture or in the activity of NS cell especially in relation to the period of exposure to the insecticide and age of the insect. The present investigation deals with cytomorphological changes caused by DDT in the neurosecretory cells of brain of adult *P. pictus*.

2. Materials and methods

Adult males and females *P. pictus* of known age were used from stock reared in the laboratory on Aak (*Calotropis*) leaves, in cages at a temperature of $28 \pm 2^\circ \text{C}$. A solution of synthetic DDT (0.01%) was obtained by dissolving a concentrated emulsion (25 E.C.) in acetone, and 0.01 ml of the solution was applied topically to the body surface just near the wing bases with a microapplicator. Controls were applied with the solvent acetone alone. For each experimental and control groups, 16 grasshoppers were used. After 24, 48 and 72 hr of incubation the grasshoppers were dissected in insects Ringer's solution and their brain fixed in aqueous Bouin's fluid. Paraffin sections ($6\mu\text{m}$) were cut and stained with Gomori's chromealum haematoxylin phloxine (CHP), paraldehyde fuchsin (PAF) (Ewen 1962) and Heidenhain's azan stain.

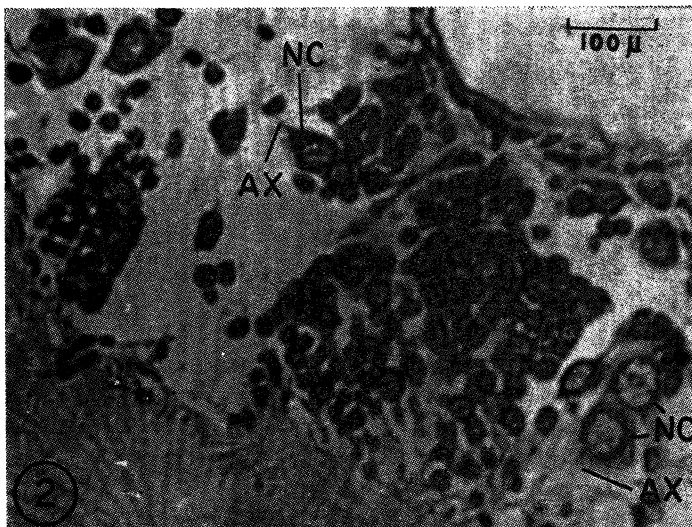
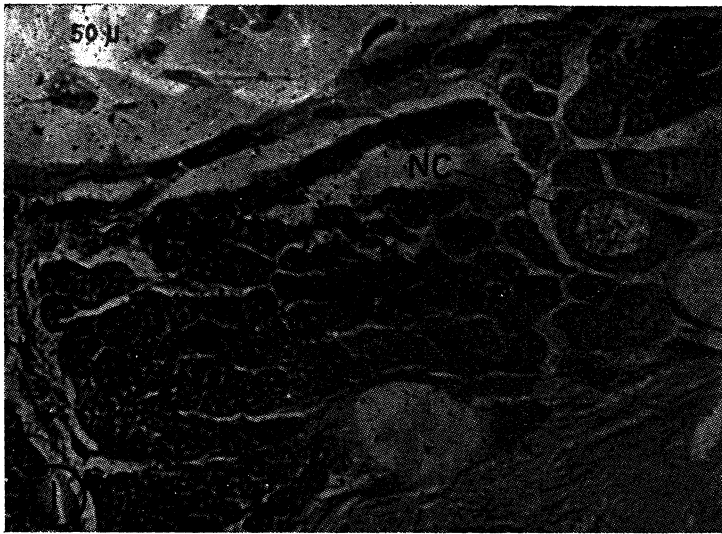
3. Results

Neurosecretory cells occur throughout the protocerebrum and tritocerebrum in different locations in the brain of *P. pictus* with the majority lying in the protocerebral lobes. On the basis of their staining reactions the cells have been classified into two types, A and B. There are two median groups of about 50–55 A type NS cells in the parsintercerebralis. A cells stain purple with PAF, dark red with Azan and dark blue with CHP. They measure $0.017 \times 0.007 \text{ mm}$, and their nuclei 0.005 mm in diameter. Occurring in the same group 30 B type cells are comparatively larger and stain green with PAF, light red with Azan and red with CHP. They measure $0.06 \times 0.02 \text{ mm}$, and their nuclei 0.01 mm in diameter. Small patches of B cells are also present dorsally, ventrally and midventrally in protocerebrum and midventrally in the tritocerebrum. The mid-brain is completely devoid of NS cells.

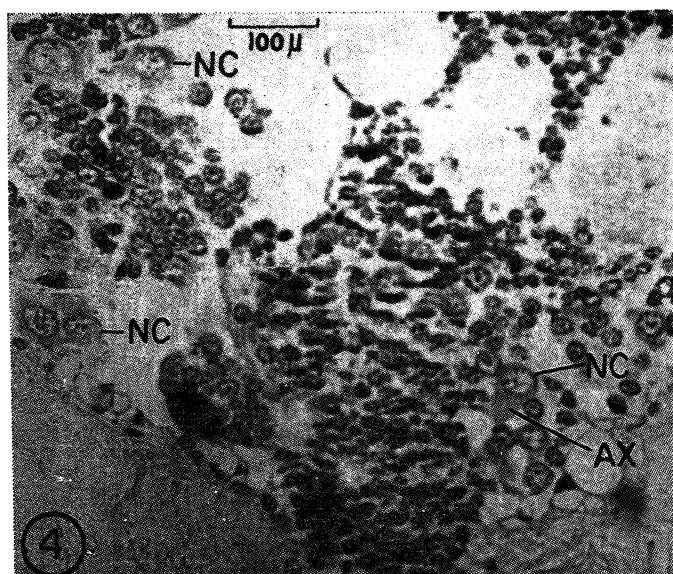
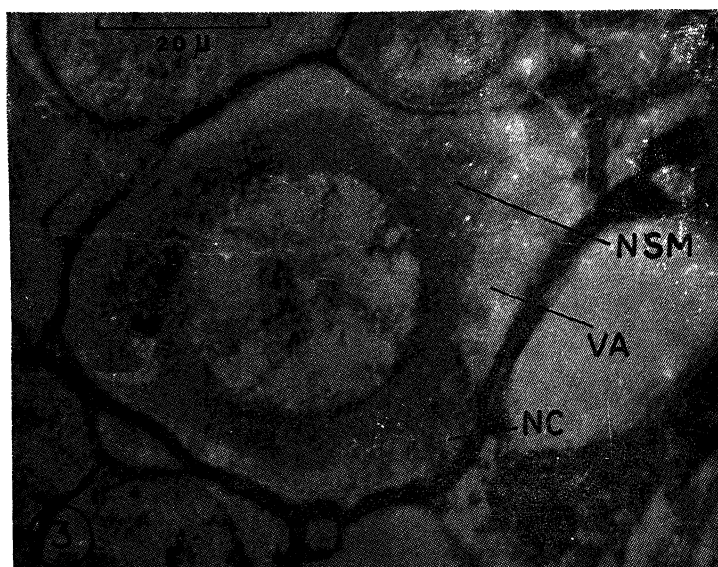
Very little neurosecretory material (NSM) is found in both A and B cells of freshly moulted adult (figure 1). Gradually the synthetic activity increases and by the time the grasshoppers mature in 5–6 days, the NS cells exhibit the peak of synthesis with deeply staining NSM in their perikarya (figure 6).

Neurosecretory cells of one day-old adult *P. pictus* treated with 0.01% DDT solution and incubated for 24 hr showed greater synthetic activity. Large quantities of NSM accumulated in the perikarya along with its simultaneous release. The release of NSM through the axons was unmistakable, as also slight undulations of the cell wall (figure 2).

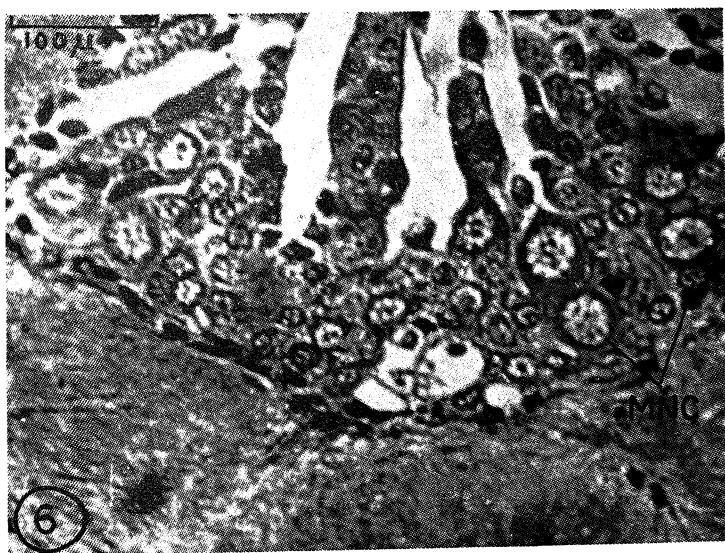
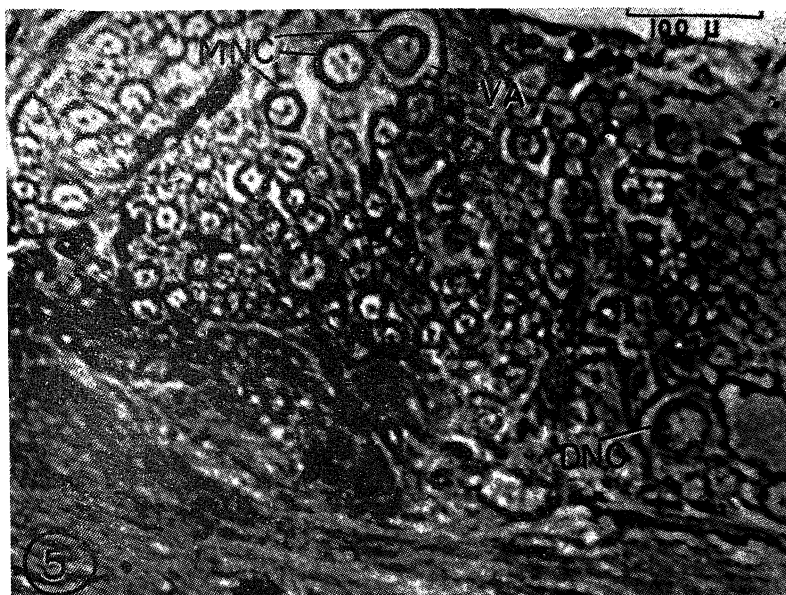
On prolonging the incubation period to 48 hr release of NSM became faster, leaving small amounts in the perinuclear region. Prolonged incubation also damaged the body of the NS cells which became polygonal or irregular in shape, apparently by the contraction of the cell wall and its infolding (figure 3). Disruption of cell wall at places was also observed (figure 4). Or further prolonging the



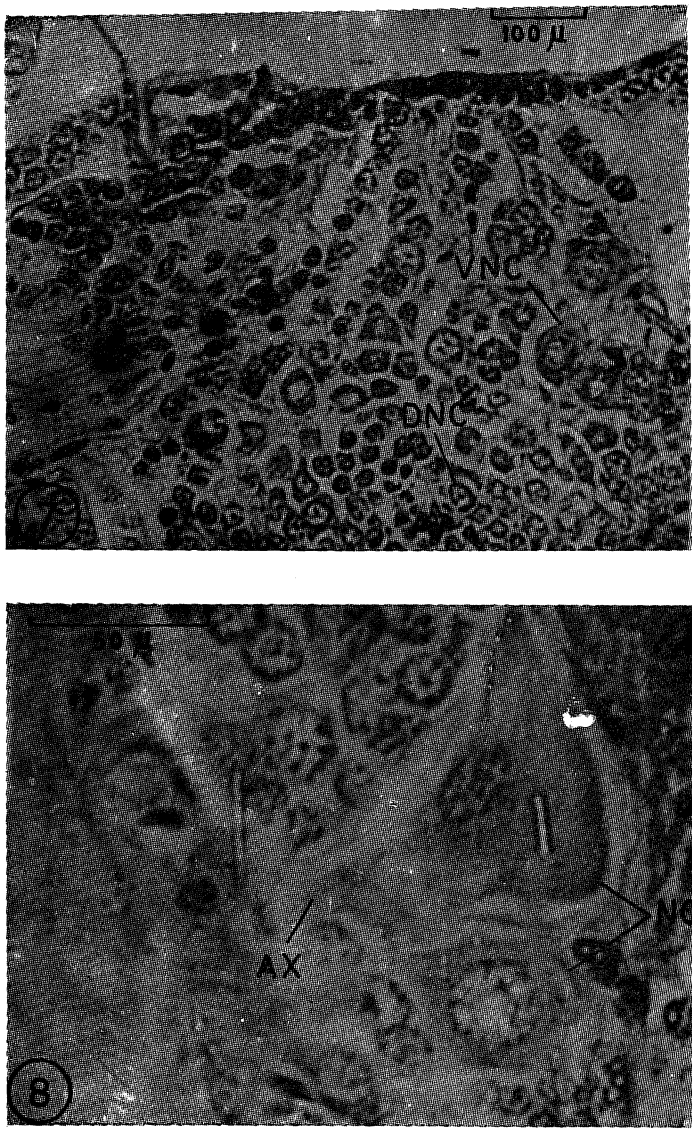
Figures 1-2. See page 309 for captions, For abbreviations see page 315.



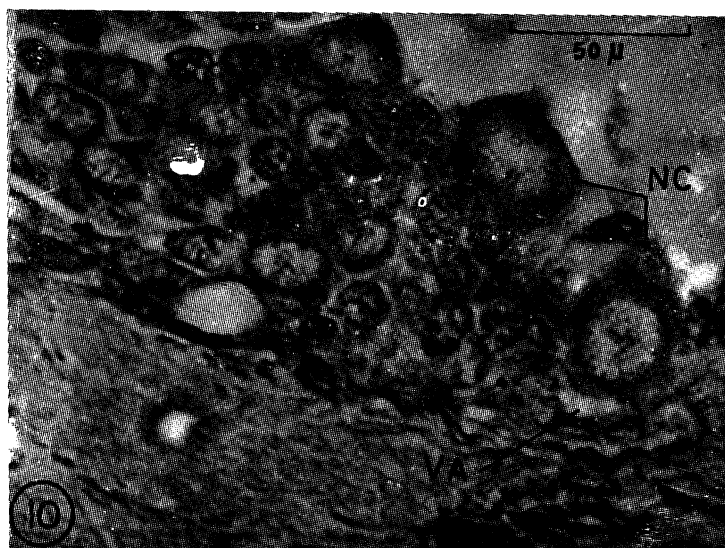
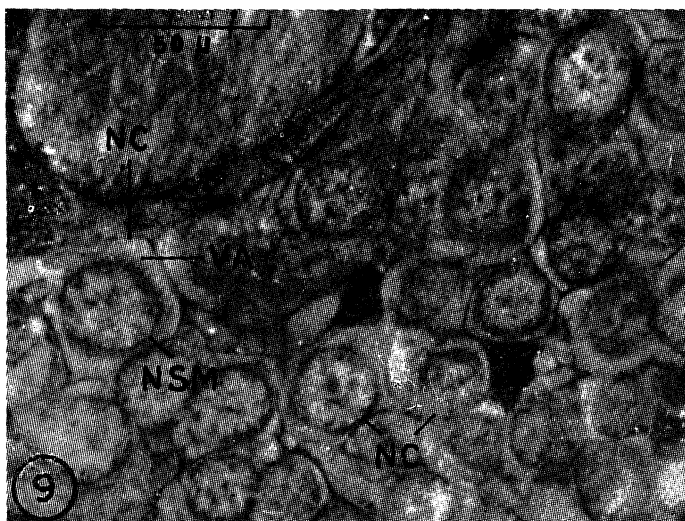
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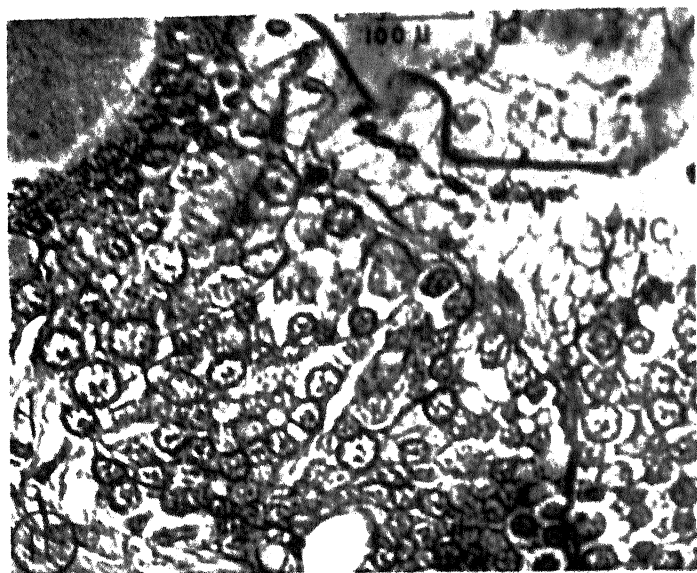
Figures 1-6. *Poekilocerus pictus*. 1. 3-day old adult showing normal neurosecretory cells. 2-5. 1-day old adult treated with 0.01% DDT for 2. 24 hr. 3, 4. 48 hr. 5. 72 hr. 6. Under control condition showing peak of synthetic activity. For abbreviations see page 315.



Figures 7-8. See page 312 for captions. For abbreviations see page 315.



Figures 9-10. See page 312 for captions. For abbreviations see page 315.



Figures 7-11. *Poecilotheres pectus*. 7-11. 6-day and 9-day old adult treated with 0.01% DDT for 7, 8, 24 hr. 9, 48 hr. 10. Under control condition. 11. 50% DDT. For abbreviations see page 315.

incubation period to 72 hr the NS cells became hyperactive in release, draining off the NSM and becoming vacuolated (figure 5).

Six-day old *P. pictus* treated with 0.01% DDT and incubated for 24 hr showed release of NSM from NS cells. Continuous release of NSM from the cell perikarya imparted the later a foamy appearance (figure 7). At this stage they stained feebly with the cell wall showing folds and the cell becoming triangular or polygonal in shape (figure 8). When the incubation period was prolonged to 48 hr, the discharge of NSM further increased and extensive damage to the NS cells was noticed in the form of undulation of cell wall and shrinkage of cell perikarya (figure 9). After 72 hr of incubation no NSM was noticed in the perikarya but some could be seen at the axonal endings (figure 11).

4. Discussion

Gundevia (1972) studied the effect of insecticides on the NS cells of insects and reported that short incubation periods of Dimecron, Diazinon and Dieldrex triggered synthetic activity of NS cells in *Hydrophilus olivaceous* Fabr. Sabesan and Ramalingam (1979) also observed increased synthetic activity in the median neurosecretory cells of endosulphon-treated *Odontopus varicornis* at the initial stage of poisoning, resulting in an accumulation of secretory material in the cell perikarya. In our studies in *P. pictus* DDT acted in the same way. The initiation of synthesis, gradual acceleration in the pace of synthetic activity and accumulation of secretory material etc., was probably an initial response to the emergency caused by the action of insecticides. Prolonged incubation, however, resulted in the discharge of secretory material. It thus seems logical that insecticidal action, up to a certain level, stimulated the synthesis and storage of secretory products but later it affected a releasing stimulus. The prolonged incubation also seems to have an inhibitory effect on synthesis and accumulation leading to large scale depletion of cellular contents and vacuolation, etc.

According to Wilcoxon and Hartzell (1933), Hartzell (1934), Richard and Cutkomp (1945) and Roche and Lhoste (1958) the motor neurones in general undergo vacuolation due to the action of insecticides. Gundevia (1972), Nanda (1974) and Sabesan and Ramalingam (1979) also made similar observations in *H. olivaceous*, *P. americana* and *O. varicornis* respectively. As neurosecretory cells are modified motor neurones, the vacuolation caused by the insecticides can be well compared to the effect observed by the above mentioned workers.

It is quite obvious from our observations that DDT produced histologically recognisable degenerative changes in the NS cells. They were in the form of undulations in the periphery of cell wall, loss in compactness, change in cell shape and sometimes even the disruption of cell wall. Similar changes have been noted in the nerve cells of insecticide treated insects by a number of workers. Wilcoxon and Hartzell (1933) and Hartzell (1934) observed trigolysis of Nissle granules and tissue disintegration in the brain nerve cells of *Tenebrio molitor* and *Melanoplus femur rubrum* after Pyrethrum and Pyrethrin treatment. Chang (1951) showed destruction of Golgi bodies and their almost complete disappearance at the time of death in the neurones of DDT-treated *P. americana* and *Apis mellifera*. Brown (1963) also noted some abnormalities in the central nervous system

of *P. americana* after treatment with Heptachlore. Various grades of disturbances in the compactness of NS elements and undulations in the periphery of cell wall after insecticidal treatment were reported by Gundevia (1972) and Nanda (1974) also. Loss in compactness, undulations towards the periphery of cell wall, tendency of the cells to become polygonal or irregular and the disruption of cell wall in the present investigation could be due to vigorous release of secretory material and loss of cohesion amongst the NS cells. The studies of Wilcoxon and Hartzell (1933), Hartzell (1934) and Chang (1951) revealed that the Golgi elements of the secretory cells became affected due to the action of insecticide. It is well-known that the Golgi bodies play a major role in the cellular secretion. Thus it can be assumed that with shorter incubation periods the Golgi bodies of secretory cells become activated and with prolonged action of the insecticide they became exhausted leading to depletion and vacuolation in the cell perikarya.

Regarding the nature of the secretory product thus released Sternberg (1963) reported that excessive stress on NS cells leads to the discharge of pharmacologically active substances. It has been shown recently that insecticidal treatment causes release of various neurohormones in insects. Maddrell and Casida (1971) treated *Rhodnius prolixus* with 42 different insecticides, of which 18 caused paralysis and release of the diuretic factor at that time. Maddrell and Reynold (1972) reported that paralytic dose of insecticides caused the release of plasticizing hormone also. Granett and Leeling (1972) on the other hand showed the appearance of hyperglycaemic agent in haemolymph, causing the trehalose content to increase in DDT-treated *P. americana*. More recently Samaranayaka (1974) reported the release of adipokinetic and hyperglycaemic hormone also in insecticide-treated *Schistocerca gregaria*.

Taking these cases as typical ones, it seems likely that it may be a general way of the action of insecticides in which they provoked a more or less simultaneous release of several, possibly all of the insect neurohormones. In this way it seems probable that such a widespread and unbalanced release of neurohormones, the controlling factors, causes serious damages to the insects and is also responsible for the lethal effect of insecticides.

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References

- Brown A W A 1963 Chemical injuries in *Insect pathology* (ed.) E A Steinhaus (New York and London : Academic Press) pp. 65-131
- Chang P I 1951 The action of DDT on the Golgi bodies of insect nervous tissue ; *Ann. Ent. Soc. Am.* **44** 311-326
- Ewen A B 1962 Histophysiology of the neurosecretory system and reterocerebral endocrine glands of the alfalfa plant bug, *Adolophocoris lineolatus* (Geuze) ; *J. Morphol.* **111** 255-269
- Ghosh J J, Ghosh S, Chanda S, Sikdar K and Bhaduri S 1968 Action of tranquillizer drugs at the level of brain nucleoprotein ; *Sci. Cult.* **34** 62
- Granett J and Leeling N C 1972 A hyperglycaemic agent in the serum of DDT-prostrate American cockroach *Periplaneta americana* ; *Ann. Entomol. Soc. Am.* **65** 299-302

- Gundevia H S 1972 *Hormonal studies of insect reproduction: Histological, histochemical and neuroendocrinological studies in relation to reproduction in Hydrophilus olivaceus* Fsbr. (Hydrophilidae—Polyphaga, Coleoptera), Ph.D. thesis, Banaras Hindu University
- Hartzell A 1934 Histopathology of insect nerve lesion caused by insecticides; *Contrib. Boyce Thompson Inst.* 6 211–223
- Maddrell S H P and Casida J E 1971 Mechanism of insecticide induced diuresis in *Rhodnius*; *Nature (London)* 231 55–56
- Maddrell S H P and Reynold S E 1972 Release of hormones in insects after poisoning with insecticides; *Nature (London)* 236 404–406
- Masner P, Hoot L, Corriveau G W and Prudhomme J C 1970 Effect of reserpine on the function of gonad and its neuroendocrine regulation in tenebrionid-beetle; *J. Insect. Physiol.* 16 2327–2344
- Matsuzawa T 1964 Quantitative chemical and pathological studies on changes of hypophysoadrenal system in response to formalin stress; in *Gunma symposia of endocrinology* Vol I 183–189
- Nanda D K 1970 Effect of Diptrex administration on the cephalic neuroglandular element of *Periplaneta americana*; *Indian J. Physiol. Allied Sci.* 24 69–79
- Nanda D K 1973 Effect of some neuropharmacological drugs on the supracerebral neurosecretory cells of *Periplaneta americana*; *Folia Biol.* 21 329–338
- Nanda D K 1974 Impact of insecticides on the brain neuroglandular elements of *Periplaneta americana*; *Die Naturwissenschaften* 61 451–452
- Prasad Om and Srivastava V K 1980 Effect of BHC on brain neurosecretory cells of *Poecilocus pictus* (Orthoptera: Acrididae); *J. Mikrosk. Anat. Forsch. Leipzig* 2, s. 250–256
- Ramade F 1967 Contribution a l'etude du mode d'action de certains insecticides du synthese plus particulierement du lindane et des phenomene de resistance a ces composes chez *Musca domestica*; *Ann. Inst. Nation. Agron. Paris* 5 11–228
- Richard A G and Cutkomp H 1945 Neuropathology in insects; *J.N.Y. Entom. Soc.* 53 313–353
- Roche A and Lhoste J 1958 Action de quelques insecticides sur les corps de Nissl des ganglions thoraciques de *Drosophila melanogaster* Meig. et de *Blatella germanica* L.; *Bull. Soc. Entomol. France* 63 181–184
- Sabesan S and Ramalingam N 1979 Effects of endosulphon on the medial neurosecretory cells of adult male *Odontopus varicornis* (Pyrrhocoridae: Heteroptera); *Entomon.* 4 223–228
- Samaranayaka A M 1974 Insecticide induced release of hyperglycaemic and adipokinetic hormone of *Schistocerca gregaria* Gen. Comp. *Endocrinol.* 24 424
- Sternburg J 1963 Autointoxication and some stress phenomena; *Annu. Rev. Entomol.* 8 19–35
- Voitkevitch A A and Leonova L K 1964 Effect of vertebrate hormones on the neurosecretory system of an insect; *Dokl. (Proc.) Acad. Sci. U.S.S.R.* 157 526–536
- Wilcoxon F and Hartzell A 1933 Some factors affecting the efficiency of contact insecticides. III. Further chemical and toxicological studies of pyrethrum; *Contrib. Boyce Thompson Inst.* 5 115–127

Abbreviations: NC, neurosecretory cell; MNC, median neurosecretory cell; DNC, damaged neurosecretory cell; VNC, vacuolated neurosecretory cell; NSM neurosecretory material; Va, vacuole; Ax, axon.

Rhythmic oscillations in non-aggressive social behaviour in *Bandicota bengalensis*

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Abstract. Non-aggressive social behaviour rhythms of *Bandicota bengalensis* were studied in the laboratory. The species exhibited 95% rhythmicity for social behaviour but the rhythms were uni- or bimodal and were influenced by the number and sex of interacting conspecifics. Peaks occurred at 0900 and 1800 hr. Males were more socially active than females.

Keywords. *Bandicota bengalensis*; social behaviour; rhythms; unimodal; bimodal.

1. Introduction

The lesser bandicoot rat, *Bandicota bengalensis*, is a widely distributed pest both in agricultural fields as well as in warehouses in India, Nepal, Burma, Thailand, Sri Lanka, Indonesia and Vietnam (Barnett and Prakash 1975). In spite of being a major rodent pest, its behaviour has not been sufficiently investigated (Spillet 1968; Parrack and Thomas 1970). Here we describe its non-aggressive social behaviour rhythms observed under laboratory conditions. Rhythms of aggressive behaviour have been reported elsewhere (Sridhara and Krishnamoorthy, in press).

2. Material and methods

2.1 Animals

Bandicota bengalensis in the weight range of 200-250 g were collected from fields by digging their burrows. On transport to laboratory the subjects were maintained in 35 × 35 × 50 cm galvanized iron mesh cages for 15 days to acclimate them to the laboratory conditions. During this period they were fed on standard rat and mouse feed (Hindustan Lever, India). Vitamins through water and fresh vegetables were made available once a week. The photoperiod was regulated at 12 hr light and 12 hr darkness with the former beginning at 0600 hr. Room temperature was 25 ± 3° C.

2.2 Behaviour studies

The non-aggressive social behaviour under different social conditions, such as confrontation between male-female, male-male, female-female, one male-two females, one male-three females, two males-one female and three males-one female was observed, and the rhythmicity for each sex was noted. The parameters of non-aggressive social behaviour were the frequency of occurrence of several acts and postures namely attend, approach, nosing, nose-nose, investigate, ano-genital sniffing, push-past, crawling under/over, huddling, allogrooming and the various sexual activities like following, attempted mount, mount, intromission, ejaculation, post-copulatory groom and lordosis. The terms and identification of behaviour patterns were based on the studies of Grant and Mackintosh (1963), Ewer (1971), Barnett (1975), Begg and Nelson (1977) and Beach (1976).

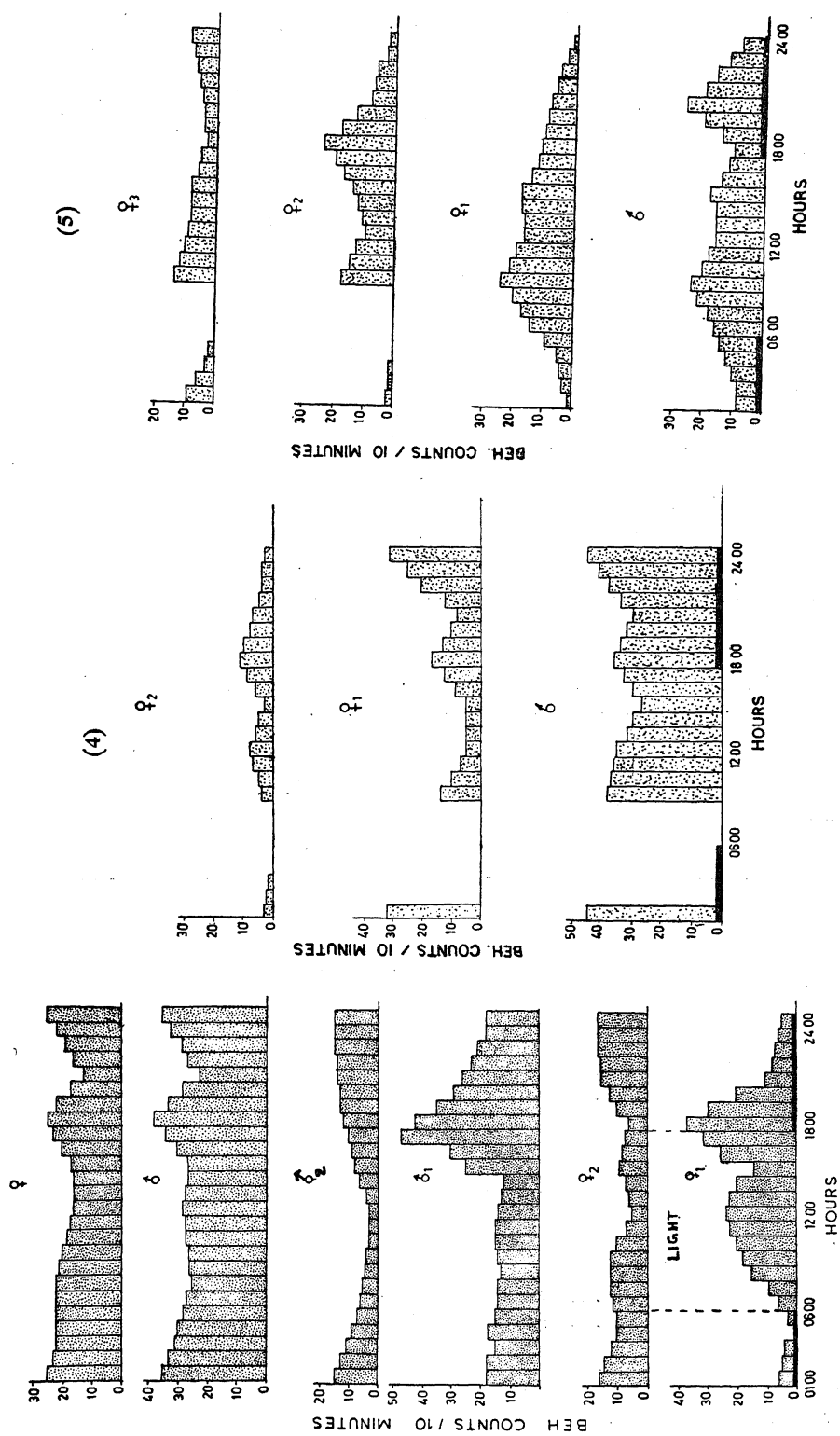
For studying behaviour the subjects were transported to a 100 × 50 × 50 cm observation chamber, made of galvanised iron with glass sides and front. The roof of the cage was made of wire mesh. The sides were of sliding type to facilitate easy introduction of animals. The chamber was divisible into two equal portions by inserting a thin galvanised iron sheet into the slot at the centre of the roof. The two sexes of different combinations were isolated in these portions for 5-6 days prior to the study to habituate them to the test chamber. During observations on social behaviour, the partition was removed for 10 minutes, the acts and postures of non-aggressive social behaviour scored for each individual during the ensuing interaction. Rhythms were established after collecting data during each hour of the L.D. cycle over a schedule of seven days. The observations were made under natural light during day-time and under dim red light during nights. The light was kept 2 M away from the cages.

2.3. Statistics

The cumulative means of each individual were computed into histograms (figures 1-5). The mean \pm S.E. of behaviour scores of the two sexes were subjected to student *t* test to establish which of the sex is more active socially in the various confrontations. For group encounters Kruskal-Wallis one way analysis of variance (Siegel 1956) was carried out to determine the rank order of sociability amongst the interacting individuals.

3. Results

Except in females of group encounters all the subjects were socially active throughout the 24 hr L.D. cycle. The results presented in figures 1-5 indicate that *B. bengalensis* exhibits rhythmic social behaviour in most of the encounters. The seven day schedule of observations indicated a fairly consistent rhythmicity (95%). The rhythms were unimodal or bimodal depending on the sex and number of interacting conspecifics. For instance, male one of male-male encounter displayed a single peak at 1800 hr (figure 2) whereas the male of one male-three females group confrontation exhibited two peaks at 0900 and 2100 hr. No definite peak was seen for the two members of male-female interaction (figure 1), male



Figures 1-5. Each histogram represents the oscillatory social behaviour of an interacting individual in the cage over a 24 hr period.

two of male-male encounter (figure 2), female two of female-female pair (figure 3) and the male of one male-two females combination (figure 4). However slightly raised social activities were seen at 1800, 2300-0100, and 0100 hr respectively for these animals. Amongst females, female one and three of one male-three females group encounter exhibited a single peak of non-aggressive social behaviour at 0900 hr (figure 5) while two peaks were seen for female one of female-female interaction at 1200 and 1800 hr (figure 3), female two of one male-two females group encounter and one male-three females group confrontation. The former had peaks of social behaviour at 1200 and 1800 hr (figure 4) and the latter at 0900 and 1800 hr (figure 5). Only one female, female one of male-two females group displayed three peaks of social behaviour rhythm at 0900, 1800 and 0100 hr (figure 4). Majority of the peaks occurred at 0900 and 1800 hr for both males and females.

Between the sexes male was more active socially in male-male ($P < 0.001$, table 1) and one male-two females encounter ($P < 0.001$, table 1). In isosexual pairs one of the pairs was significantly more social ($P < 0.001$ and $P > 0.05$, table 1). The rank order of social behaviour in one male-two females group encounter was male $>$ female one $>$ female two ($H = 7.93$, $P > 0.05$) while there was no such hierarchy in the one male-three females interaction ($H = 3.21$, $P < 0.05$). However, female one was more sociable than female three (t test

Table 1. Comparison of non-aggressive behaviour scores of male and female *B. bengalensis* during different social conditions.

Confrontation between	Scored by	Mean beh. counts \pm SE	Cumulative score for the combination
Male and female	Male	1393 \pm 47*	2352
	Female	959 \pm 42	
Male and male	Male one	1024 \pm 31*	1457
	Male two	433 \pm 52	
Female and female	Female one	733 \pm 49*	1247
	Female two	504 \pm 49	
One male and two females	Male	1187 \pm 98*	1819
	Female one	418 \pm 26	
	Female two	220 \pm 21	
One male and three females	Male	190 \pm 23	821
	Female one	281 \pm 26**	
	Female two	213 \pm 28	
	Female three	137 \pm 14	

* Significantly higher social activity score.

** More social than female three.

$P < 0.01$, table 1). When group encounters involved more than one male, social behaviour was least but aggression was so violent that all males except the dominant were found dead much before the 24 hr cycle. The death seemed to be due to the stress of constant threats and fighting rather than due to injuries since none of the dead males was fatally wounded.

4. Discussion

Several species of rodents exhibit well-established rhythms for movement outside their burrows in nature (Marten 1976), activity and movement in cages and maize (Barnett *et al* 1975), and for aggressive behaviour (Lerwill 1977). These studies showed two peaks of activity for rats, one soon after dark period and a second before dawn with little activity during the day-time. A similar bimodal rhythmicity for locomotor activity was observed in *B. bengalensis* by Parrack (1966). Agonistic behaviour of a group of lesser bandicoots did not exhibit any rhythms; however, it tended to be increased between 0200 and 0530 hr (Spillet 1968). Parrack and Thomas (1970) observed dominant *B. bengalensis* exhibiting peak aggressive behaviour at 0600 and 0900 hr. This period coincided with the visit of subordinate rat to the food platform. The present results also demonstrate that non-aggressive social behaviour of *B. bengalensis* also manifests both uni- and bimodal rhythms depending on the sex and number of conspecifics interacting. Similar alteration of rhythms in response to social interaction has been demonstrated for several species of rodents. For instance, Kavanau (1967) observed synchronization of running between female deer mice, while Calhoun (1975) noticed alteration of locomotor activity by sexual rhythms in Norway rats. Our earlier study showed circadian oscillations in the aggressive behaviour of *B. bengalensis* consequent on social stress (Sridhara and Krishnamoorthy, in press). Farr and Andrews (1978) described the phase dissociation of both metabolic and behavioural rhythms of deer mice, *Peromyscus maniculatus* when crowding increased social interactions. The phase relationships of subordinate subjects was prone to be much more unstable which the authors attribute to their avoidance of more aggressive and dominant cohorts. The fluctuation in the social behaviour rhythms seen in the present study also could be due to the degree of stress experienced by the subordinate animals during the various social encounters staged.

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References

- Barnett S A 1975 *The rat ; A study in Behavior* Chicago : The University of Chicago press)
- Barnett S A, Cowan P E and Prakash I 1975 Circadian rhythms of movements of the house rat, *Rattus rattus* L. ; *Indian J. Exp. Biol.* 13 153-155
- Barnett S A and Prakash I 1975 *Rodents of economic importance in India* (New Delhi : Arnold Heineman)

- Beach F A 1976 Sexual behavior in male rat; *Science* **153** 769-770
- Begg R J and Nelson J B 1977 The agonistic behavior of *Rattus villosissimus*; *Aust. J. Zool.* **25** 291-327
- Calhoun J B 1975 Social modifications of activity rhythms in rodents; *Chronobiol. Suppl.* **1** 11
- Ewer R F 1971 The biology and behavior of a free living population of black rats; *Anim. Behav. Monogr.* **4** 127-174
- Farr I and Andrews R V 1978 Rank associated desynchronization of metabolic and activity rhythms of *Peromyscus maniculatus* in response to social pressure; *Comp. Biochem. Physiol.* **A61** 539-542
- Grant E C and Mackintosh J H 1963 A comparison of the social postures of some common laboratory rodents; *Behaviour* **21** 246-259
- Kavanau J L 1967 Behaviour of captive white footed mice; *Science* **155** 1623-1639
- Lerwill C 1977 Diurnal variations in the agonistic behaviour of the golden hamster (*Mesocricetus auratus*); *J. Zool.* **182** 159-167
- Marten P 1976 The patterns of *Peromyscus* activity and their correlation with weather; *J. Mammal.* **54** 169-188
- Parrack D W 1966 The activity cycle of the lesser bandicoot rat, *Bandicota bengalensis*; *Curr. Sci.* **35** 544-545
- Parrack D W and Thomas J 1970 The behaviour of the lesser bandicoot rat, *Bandicota bengalensis* (Gray and Hardwicke); *J. Bombay Nat. Hist. Soc.* **67** 67-80
- Siegel S 1956 *Non-parametric statistics for the behavioural sciences* (New York : McGraw-Hill)
- Spillet J J 1968 *The ecology of the lesser bandicoot in Calcutta* (Bombay : Bombay Natural History Society)
- Sridhara S and Krishnamoorthy R V 1982 (in press) Oscillatory aggressive behaviour of captive *Bandicota bengalensis*. To be published in the *Proceedings of National Seminar on Rodent Research and Training*, Central Arid Zone Research Institute, Jodhpur.

Toxicity of certain pesticides found in the habitat to the larvivorous fishes *Aplocheilus lineatus* (Cuv. & Val.) and *Macropodus cupanus* (Cuv. & Val.)

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Abstract. Bioassay studies reveal the toxicity levels of pesticides utilised in the area to the larvivorous fishes *Aplocheilus lineatus* and *Macropodus cupanus*. The resistance of both fishes decreases with increase in period of exposure to the pesticides. Comparing the major groups of synthetic organic pesticides, the chlorinated hydrocarbons, here exemplified by DDT, are more toxic to the fishes than ekalux and malathion, the organophosphates experimented with. The carbamate sevin is the least toxic. Nevertheless, all the pesticides are 'toxic' to 'very toxic' as defined by the Joint ICMO/FAO/UNESCO/WHO group of experts, having an acute lethal threshold of below 1 to 100 mg/l. *M. cupanus* is the more resistant of the two fishes, probably on account of its obligate air-breathing nature, and thus its tendency to absorb less toxicant across the gills. Contrasting the susceptibility of mosquito larvae and the fishes studied to the pesticides investigated, the closeness of the LC_{50} values obtained in *A. lineatus* to that recorded in certain species of mosquito larvae indicates that while *M. cupanus* could be employed in conjunction with pesticides for anti-larval work, *A. lineatus* should not be so utilised.

Keywords. Pesticides ; toxicity ; larvivorous fish ; *Aplocheilus lineatus* ; *Macropodus cupanus*.

1. Introduction

Larvivorous fishes such as *Gambusia affinis* and *Poecilia reticulata*, the primary biological control agents of mosquito larvae, have been extensively employed in certain regions in mosquito abatement programmes (Mallars and Fowler 1970; Bay and Self 1972). However, indiscriminate releases of these exotics into the aquatic environment has resulted in the alteration/eradication of valuable faunal components of the ecosystem (Myers 1965; Bay 1973; Menon 1977). This has renewed interest in the biocontrol potential of indigenous larvivorous fishes such as *Aplocheilus lineatus* (Cuv. & Val.) and *Macropodus cupanus* (Cuv. & Val.). An essential aspect of such assessments is information on the danger levels to the fishes of pesticide contaminants found in the aquatic ecosystem. This problem has assumed importance owing to the widespread and indiscriminate permeation

of pesticides in the aquatic environment (Muirhead-Thomson 1971; Edwards 1977) and the consequent risks to larvivoracious fish populations. Such data are not available, leading to this study.

2. Materials and methods

In the present investigation, pesticides were chosen from each of the major groups of synthetic pesticide utilised in agricultural operations in the area—i.e., DDT (25 EC; manufactured by Bangalore Pesticides Limited) from the chlorinated hydrocarbons, malathion (50 EC; manufactured by Bangalore Pesticides Limited) and ekalux (25 EC; manufactured by Sandoz India Limited) from the organophosphates, and sevin (50% WP; manufactured by Union Carbide) from the carbamates and bioassay tests were conducted.

Healthy medium sized *A. lineatus* (mean standard length 25–40 mm) and *M. cupanus* (mean standard length 20–28 mm) collected from streams and water bodies in the Trivandrum (Kerala, South India) area were acclimated to laboratory conditions in well water at a temperature of $28 \pm 2^\circ \text{C}$, pH of 7.1 and O_2 at near air saturation. The static test method (Doudoroff *et al* 1951) was used to directly estimate the toxicity levels, with certain modifications to guard against a depletion/alteration in the toxic material, as suggested by Muirhead-Thomson (1971) and Sprague (1973). Stock solutions of the different pesticides were diluted to the required parts by weight of active ingredient (= mg/l) by standard methods (Busvine 1977). However, since the water volume/weight of fish ratios utilised for bioassay tests vary greatly (Rita and Nair 1978), here, on the basis of preliminary trials, 1.8 gm/l solution and 1 gm/l solution were chosen as an adequate weight/volume ratio in *A. lineatus* and *M. cupanus*, respectively. Bioassays were carried out in 5 logarithmic concentrations. The period of exposure for each bioassay was 48 hr as subsequently the mortality curve flattened; neither the experimental nor control specimens were fed during this period. The lethal concentration 50 (LC_{50}) for 24 and 48 hr were calculated for each pesticide by the probit analysis method. The behavioural responses exhibited by the fishes during the exposure period were also recorded.

3. Results and discussion

A comparative statement of the results of the probit analysis, specifically regression equations and the LC_{50} values including the upper and lower limits (ULC_{50} and LLC_{50}) has been tabulated for both the 24 and 48 hr period of exposure in the case of each pesticide in tables 1 and 2.

Considering the physical reactions of the fish to the toxic solutions, in all cases undulation (mild to pronounced) of the body, increased oscillation of the pectoral, pelvic, anal and caudal fins, rapid and irregular movements of the opercular folds, loss of equilibrium (ranging from partial to complete) and excitation (mild to pronounced) were noted. At extremely toxic concentrations, the external body surface showed 'burnt' patches.

The lowering in the 48 h LC_{50} values when compared with the 24 hr ones suggests the decreasing resistance of the fish with increase in experimental time, a finding supported by Cairns and Scheier (1964) and Rita and Nair (1978).

Table 1. Acute toxicity levels of selected pesticides in *A. lineatus*.

Pesticide	Period of exposure (hrs)	LC ₅₀ values (mg/l)	Regression equation
DDT	24	0.1489±0.0212	$\log y = 9.5405 \cdot \log x \times 100 - 6.1893$
	48	0.1228±0.0182	$\log y = 8.0885 \cdot \log x \times 100 - 3.8103$
Ekalux	24	0.1939±0.0247	$\log y = 10.2105 \cdot \log x \times 100 - 8.1467$
	48	0.1699±0.0238	$\log y = 9.6205 \cdot \log x \times 100 - 6.8348$
Malathion	24	1.1560±0.3050	$\log y = 5.0873 \cdot \log x \times 10 - 0.3972$
	48	0.9750±0.2130	$\log y = 6.1911 \cdot \log x \times 10 - 1.1228$
Sevin	24	4.2670±0.3750	$\log y = 14.3413 \log \cdot - 3.9490$
	48	3.7470±0.3100	$\log y = 14.6842 \log \cdot - 3.4242$

Table 2. Acute toxicity levels of selected pesticides in *M. cupanuis*.

Pesticido	Period of exposure (hrs)	LC ₅₀ values (mg/l)	Regression equation
DDT	24	2.813±0.453	$\log y = 8.6338 \cdot \log x + 1.1219$
	48	2.277±0.310	$\log y = 9.8746 \cdot \log x + 1.4720$
Ekalux	24	3.659±0.434	$\log y = 11.454 \cdot \log x - 1.4533$
	48	3.453±0.584	$\log y = 7.7358 \cdot \log x + 0.8363$
Malathion	24	4.962±0.479	$\log y = 13.2989 \cdot \log x - 4.2607$
	48	4.594±0.557	$\log y = 10.5503 \cdot \log x - 1.9859$
Sevin	24	14.730±0.590	$\log y = 35.2288 \cdot \log x - 36.1552$
	48	13.910±0.380	$\log y = 44.0285 \cdot \log x - 45.3320$

The higher LC₅₀ values in *M. cupanuis* denote its greater resistance than *A. lineatus*. This may be because the principal route of entry of pesticides for non-feeding fish is through the gills (Johnson 1968); *M. cupanuis*, being an obligate air-breather, naturally tends to absorb less toxicant across the gills. Comparing the main groups of synthetic organic pesticides, the results of the present study where DDT (a chlorinated hydrocarbon) is more toxic to the fish than ekalux, malathion (organophosphates) and sevin (a carbamate), are in agreement with the findings of Johnson (1968) and Rita and Nair (1978). However, all pesticides tested are 'toxic' to 'very toxic' as defined by the Joint ICMO/FAO/UNESCO/WHO group of experts (1964) since they have an acute lethal threshold of below 1 to 100 mg/l. A comparison of the acute toxicity levels of the pesticides in various species of fishes, given in table 3, reveals that wide variations in the

Table 3. Comparison of some acute toxicity levels of the pesticides investigated in different species of fishes.

Pesticide	Species investigated	Period of exposure (hrs)	LC ₅₀ (mg/l) (ppm)	Reference
DDT	<i>Lepomis macrochirus</i>	96	0.016	Edwards (1977)
	<i>Salmo gairdneri</i>	96	0.018	Edwards (1977)
	<i>Salvelinus fontinalis</i>	36	0.0323	Hatch (1957)
	<i>Carassius auratus</i>	72	0.1	Odum and Summerford (1946)
	<i>Carassius auratus</i>	96	0.027	Henderson <i>et al</i> (1959)
	<i>Aplocheilus lineatus</i>	24	0.1489	Present investigation
	<i>Aplocheilus lineatus</i>	48	0.1228	Present investigation
	<i>Gambusia affinis</i>	24	0.5	Mayhew (1955)
	<i>Gambusia affinis</i>	36	0.32	Hatch (1957)
	<i>Gambusia affinis</i>	72	0.01	Odum and Summerford (1946)
	<i>Macropodus cupanus</i>	24	2.813	Present investigation
	<i>Macropodus cupanus</i>	48	2.277	Present investigation
	<i>Puntius ticto</i>	24	0.0135	Bhatia (1971)
	<i>Puntius ticto</i>	48	0.011	Bhatia (1971)
	<i>Puntius ticto</i>	72	0.011	Bhatia (1971)
	<i>Puntius ticto</i>	96	0.0074	Bhatia (1971)
	<i>Salmo gairdneri</i>	96	0.1	Edwards (1977)
	<i>Lepomis macrochirus</i>	96	0.12	Edwards (1977)
	<i>Aplocheilus blochii</i>	48	1.3	VCRC Annual Report (1979)
Malathion	<i>Aplocheilus lineatus</i>	24	1.15	Present investigation
	<i>Aplocheilus lineatus</i>	48	0.975	Present investigation
	<i>Cyprinus carpio</i>	96	4.5	Nishiuchi and Hoshimoto (1967)
	<i>Macropodus cupanus</i>	24	4.962	Present investigation
	<i>Macropodus cupanus</i>	48	4.594	Present investigation
	<i>Labeo rohita</i>	24	7.15	Arora <i>et al</i> (1971)
	<i>Labeo rohita</i>	96	5.05	Arora <i>et al</i> (1971)
	<i>Pimephales promelas</i>	24	25	Tarzwel (1958)
	<i>Pimephales promelas</i>	96	12.5	Henderson <i>et al</i> (1959)
	<i>Pimephales promelas</i>	96	22	Tarzwel (1958)
	<i>Lepidocephalus thermalis</i>	24	22.69	Rita (1977)
	<i>Lepidocephalus thermalis</i>	48	20.61	Rita (1977)
	<i>Oncorhynchus kisutch</i>	96	0.7	Macek and McAllister (1970)
	<i>Ameiurus melas</i>	96	0.8	Macek and McAllister (1970)
	<i>Fundulus similis</i>	24	1.75	Butler (1963)
	<i>Lepomis macrochirus</i>	96	2.0	Henderson <i>et al</i> (1959)
	<i>Lepomis macrochirus</i>	96	3.4	Edwards (1977)
	<i>Salmo gairdneri</i>	96	3.5	Edwards (1977)
Sevin	<i>Aplocheilus lineatus</i>	24	4.207	Present investigation
	<i>Aplocheilus lineatus</i>	48	3.747	Present investigation
	<i>Mugil curema</i>	24	4.25	Butler (1963)
	<i>Perca flavescens</i>	96	5.6	Macek and McAllister (1970)
	<i>Casterosteus aculeatus</i>	24	6.7	Stewart <i>et al</i> (1967)
	<i>Pimephales promelas</i>	96	13	Henderson <i>et al</i> (1959)
	<i>Macropodus cupanus</i>	24	14.73	Present investigation
	<i>Macropodus cupanus</i>	48	13.91	Present investigation

pesticide concentrations that produce adverse effects have been recorded, depending on the species, environmental factors and even biological status and origin of the test organism. It must however be mentioned that the LC_{50} values obtained for *A. lineatus* exposed to malathion are comparable to those recorded in the related *Aplocheilichthys blochii* (VCRC Annual Report 1979). Again, in the case of specimens exposed to DDT, *M. cupanus* is even hardier than the 'resistant' mosquito fish *G. affinis* (Johnson 1968). Again, *M. cupanus* is the most resistant of all the species studied to sevin.

It may also be noted that Das and Rajagopalan (1976) working on the susceptibility of mosquito larvae to insecticides found that in *Anopheles stephensi*, *Culex fatigans*, *Anopheles culicifacies* and *Aedes aegypti*, the critical doses of malathion required were 0.8, 0.064, 0.08 and 0.48 mg/l respectively. In the case of sevin it was a uniform 4.0 mg/l. With DDT, the LC_{50} value was 0.2 mg/l for *A. stephensi* and *C. culicifacies* while in the case of *Ae. aegypti* and *C. fatigans* it was 0.02 mg/l and 0.03 mg/l, respectively (VCRC Annual Report 1979). These values are fairly close to those reported in *A. lineatus* in the present study. Therefore, while *M. cupanus* could be utilised in conjunction with such insecticides for anti-larval work, *A. lineatus* should not be so used under any circumstances.

It has thus been demonstrated that even 'safe' and often minute dosages of pesticides are highly toxic to fish life, as may be seen from the LC_{50} values. Therefore, studies of this nature are essential as they provide information on the concentrations of environmental contaminants that cannot be tolerated by fish populations and consequently aid not only in the effective control of mosquito larvae by the fish but also in the protection of the aquatic environment.

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References

- Arora H C, Shrivastava S K and Seth A K 1971 Bioassay studies of some commercial organic insecticides—Parts I and II; *Indian J. Environ. Health* 13 226–233; 300–306
- Bay E C 1973 Exotic fish introductions for mosquito control: possible and purported consequences; *WHO/VBC/WP/73*
- Bay E C and Self L S 1972 Observations on the guppy *Poecilia reticulata* Peters in *Culex pipiens fatigans* breeding sites in Bangkok, Rangoon and Taipei; *Bull. World Health Org.* 46 407–416
- Bhatia H L 1971 Toxicity of some pesticides to *Puntius ticto* (Hamilton); *Sci. Cult.* 37 548
- Busvine J R 1977 *A critical review of the techniques for testing insecticides* (London: Commonwealth Institute of Entomology)
- Butler P A 1963 Commercial fishery investigation; *Pesticide-Wildlife Studies U.S. Fish. Wild. Serv. Cir.* 199 5–28
- Cairns J and Scheier A 1964 The effect upon the pumpkinseed sunfish *Lepomis gibbosus* (Linn.) of chronic exposure to lethal and sublethal concentrations of dieldrin; *Notul. Nat.* p. 370
- Das P K and Rajagopalan P K 1976 Susceptibility of larvae of *Culex fatigans* (Wiedmann), *Anopheles stephensi* (Liston) and *Aedes aegypti* (Linn.) to insecticides in Pondicherry; *Indian J. Med. Res.* 70 412–416

- Doudoroff P, Anderson B G, Burdick G E, Galtsoff P S, Hart W B, Patrick R, Strong E R, Surber E W and Van Horn W M 1951 Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish ; *Sewage Ind. Wastes* **23** 130-139
- Edwards C A 1977 Nature and origins of pollution of aquatic systems by pesticides ; In *Pesticides in aquatic environments* (ed.) M A Q Khan. (New York : Plenum Press) pp. 11-36
- Hatch R W 1957 Relative sensitivity of salmonids to DDT ; *Prog. Fish-Cult.* **19** 89-91
- Henderson C, Pickering Q H and Tarzwell C M 1959 Toxicity of organic phosphorous and chlorinated hydrocarbon insecticides to fish ; *Biol. Prob. Water Poll. Trans.* 1959 *Seminar Robt. A. Taft San. Eng. Center Tech. Rep. W.* **60-3** 76-92
- Johnson D W 1968 Pesticides and fish—a review of selected literature ; *Trans. Am. Fish. Soc.* **97** 398-424
- Joint ICMO/FAO/UNESCO/WHO group of experts 1964 Abstract of the 1st report of the 1st session on the scientific aspects of marine pollution ; *Water Res.* **3** 995-1005
- *Macek K J and McAllister W A 1970 *Trans. Am. Fish. Soc.* **99** 20
- Mallars J L and Fowler J R 1970 *Mosquito eating fishes in California* (California : Calif. Mosquito Control Ass. Inc.).
- Mayhew J 1955 Toxicity of 7 different insecticides to rainbow trout, *Salmo gairdneri* Richardson ; *Proc. Iowa Acad. Sci.* **62** 599-606
- Menon A G K 1977 Fish and malaria control ; *Sci. Cult.* **43** 110-114
- Muirhead-Thomson R C 1971 *Pesticides and freshwater fauna* (New York : Academic Press)
- Myers G S 1965 *Gambusia*, the fish destroyer ; *Trop. Fish. Hobbyist* **31-32** 54-55
- Nishiuchi Y and Hoshimoto Y 1967 Toxicity of pesticide ingredients of some freshwater organisms ; *Botyn. Kagaku* **32** 5-11
- Odum E P and Summerford W T 1946 Comparative toxicity of DDT and four analogs to goldfish, *Gambusia* and *Culex* larvae ; *Science* **104** 480-482
- Rita Kumari S D and Nair N B 1978 Toxicity of some insecticides to *Lepidocephalus thermalis* (Cuv. & Val.) ; *Proc. Indian Natl. Sci. Acad.* **44** 122-132
- Sprague J B 1973 The ABC's of pollutant bioassay using fish ; *Biological methods for the assessment of water quality ASTM, STP 528, American Society for Testing and Materials* pp. 6-30
- Stewart N E, Milleran R E and Breese W P 1967 Acute toxicity of the insecticide sevin and its hydrolytic product 1-naphthol to some marine organisms ; *Trans. Am. Fish. Soc.* **96** 25-30
- Tarzwell C M 1958 The toxicity of some organic insecticides to fishes ; *Proc. Conf. S.E. Ass. Game Fish Com.* **12** 232-239
- Vector Control Research Centre 1979 Annual Report (New Delhi : ICMR)

*Not referred to in the original

Histochemical studies on non-specific esterases in epididymis of the bat, *Cynopterus sphinx sphinx*

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Abstract. Non-specific esterases were studied in the epididymis of the bat, *C. sphinx sphinx* by employing α -naphthyl acetate and 5-bromoindoxyl acetate as substrates and eserine sulphate (10^{-4} M) as an enzyme inhibitor. The enzyme activity in epididymal cells was diffused cytoplasmic and granular in nature. The granular activity was eserine resistant. Holocrine cells were also observed in the epididymis of this bat. Seasonal variations in epididymal esterases are described.

Keywords. Bat; *Cynopterus sphinx sphinx*; epididymis; principal cells; holocrine cells; esterases.

1. Introduction

Several lysosomal acid hydrolases have been studied in the gonads and non-gonadal sex accessories of the vertebrates. Although esterases have been demonstrated in the epididymis of rat (Verne and Hebert 1952; Wachstein *et al* 1961; Vogel 1967), mouse (Allen and Slater 1957; Kirkeby and Blecher 1978a, b; Blecher and Kirkeby 1978; Chakraborty *et al* 1975), marmoset (Miraglia *et al* 1970), bull (Erkmann 1971) and man (Malaty and Bourne 1954; Kohl 1968), these enzymes have not been studied in bats.

The presence of two types of cells, viz., principal cells and holocrine cells has been reported in the epididymis of some animals. Esterases have been studied in the holocrine cells of some animals such as rat (Vogel 1967) and mouse (Martan and Allen 1964). In this regard nothing is known about the holocrine cells in the bat epididymis.

The present paper deals with non-specific esterases in epididymis of the bat *C. sphinx sphinx*.

2. Material and methods

The adult male bats (*C. sphinx sphinx*) were collected monthly for one year. The animals were killed by decapitation, the epididymides were dissected out and

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fixed in cold (4° C) Baker's fixative. Following fixation (24 hr) the tissues were transferred to Holt's gum sucrose (Holt 1959). The sections were cut at 6–8 μ m on a Lipshaw cryostat at –20° C. Before incubation, the sections were washed with chilled distilled water.

The following two histochemical techniques were employed for enzyme localization :

(i) α -naphthyl acetate (α NA) as a substrate with Fast Blue B as a coupler (Gomori 1952). (ii) 5-Bromoindoxyl acetate (5 BIA) as a substrate with ferri-ferrocyanide as an oxidizing agent (Holt 1958 ; Holt and Withers 1958).

In both the histochemical techniques, eserine sulphate (10^{-4} M) was used as an enzyme inhibitor.

3. Observations

3.1. Sex-cycle of the bat

C. sphinx sphinx is a megachiropteran frugivorous bat, the females of which experience two pregnancies in quick succession. The first pregnancy lasts from November to March and the second from March–April to July (Mote 1981). Ramakrishna (1947), Baile (1976) and Pawar (1976) also reported on two sex-cycles in this species of bat. The sex cycle in male bats may be summarized as :

Sexual quiescence—May to August ; Prebreeding period—September and October ; First active breeding period—November ; Intervening period—December and January ; Second active breeding period—February–March ; Postbreeding period—April.

Figures 1–8. (1) Epididymis during sexual quiescence (June) stained with α NA technique to show diffused cytoplasmic staining in epithelial cells (arrows). CT = connective tissue $\times 50$. (2) Early prebreeding period (September) stained with 5 BIA technique. Note diffused cytoplasmic and granular staining in the principal cells (PC), holocrine cells (HC) and fibroblast-like cells in connective tissue (FC) $\times 75$. (3) Early prebreeding period (September) stained with 5 BIA after eserine treatment. Note eserine resistant granular activity in the principal cells (PC) and holocrine cells. (arrows) $\times 90$. (4) Late prebreeding period (October) stained with 5 BIA. Note diffused cytoplasmic and granular activity in the principal cells (PC) and holocrine cells (HC). Arrows indicate few spermatozoa in the lumen $\times 90$. (5) Active breeding period (November) stained with α NA technique to show diffused cytoplasmic and granular staining in the principal cells (PC) and holocrine cells (HC). Note acrosomes of the sperms (arrows) also appear granular $\times 90$. (6) Intervening period (January) stained with α NA technique showing slight reduction in the enzyme activity in the principal cells (PC) and holocrine cells (HC). Arrows indicate sperm heads $\times 150$. (7) Postbreeding period (April) stained with 5 BIA. Note increase in the diffused cytoplasmic and granular staining in the principal cells (PC) and holocrine cells (HC). Arrows indicate a few spermheads $\times 150$. (8) Adjacent section as in figure 7 stained with 5 BIA after eserine treatment to show eserine resistant granular staining in the holocrine cells (HC) and acrosomes of the sperms (arrows) $\times 150$.

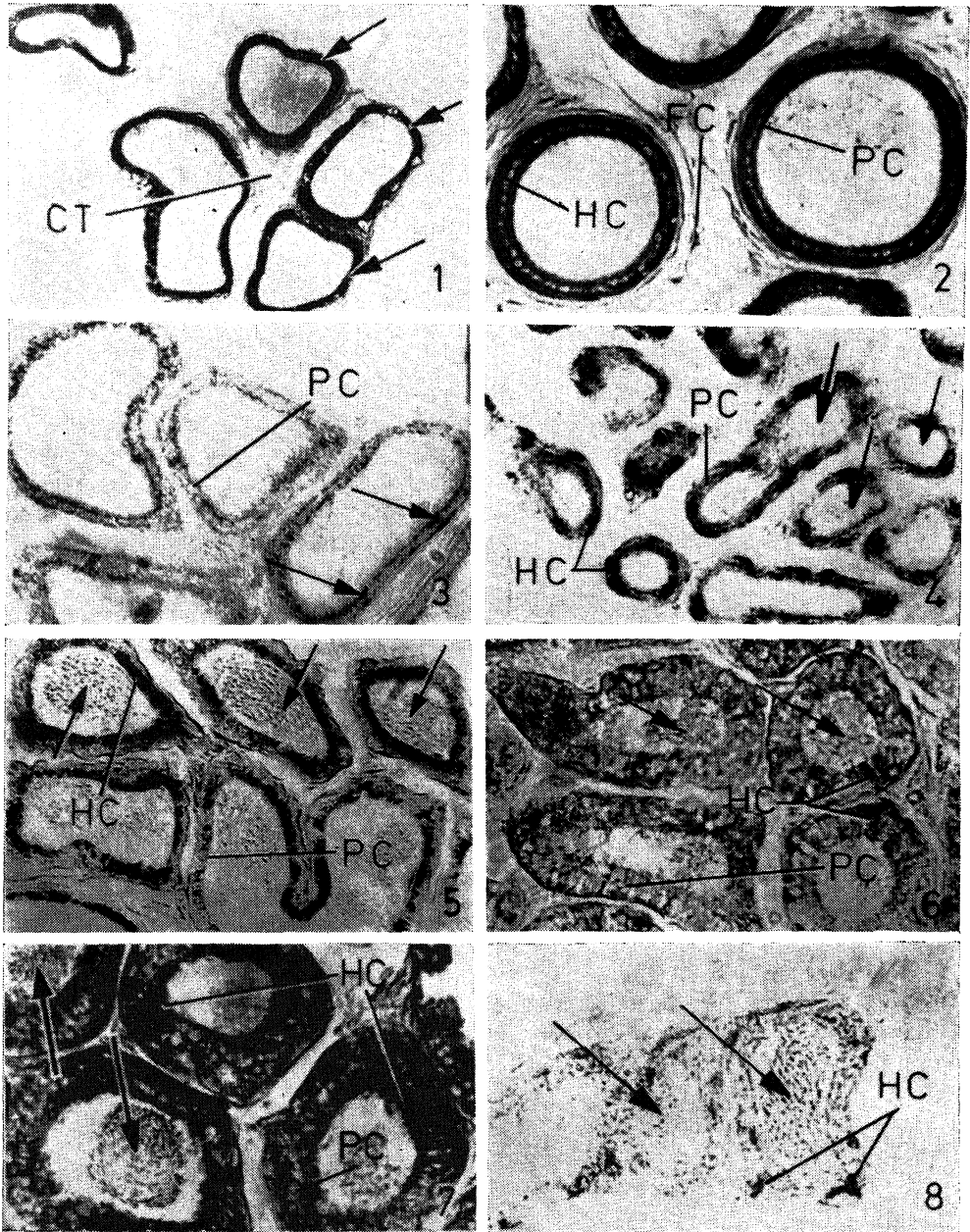


Figure 1-8.

3.2. Enzyme localization

The enzyme activity in the epithelial cells appeared as diffused cytoplasmic and in the form of granules (figure 2). The granular staining in the cells was eserine resistant (figure 3), whereas the diffused cytoplasmic staining was eserine sensitive.

3.3. Seasonal variations in esterases

The regressed epididymis during the sexual quiescence exhibited weak diffused cytoplasmic staining in the epithelial cells with α NA (figure 1) and 5 BIA. The staining was eserine-sensitive and the holocrine cells could not be distinguished from the low cuboidal principal cells. With the advent of the prebreeding period two cell types could be identified with α NA and 5 BIA (figure 2) procedures. The principal cells exhibited moderate staining which was diffused cytoplasmic and granular in nature. The holocrine cells also exhibited dual localization of enzyme activity but the staining was more intense than the principal cells. The granular staining was eserine-resistant (figure 3). Similar results were also seen during the late prebreeding period (figure 4). During this period the spermheads (acrosomes) also exhibited eserine-resistant enzyme activity.

During both the active breeding periods the principal cells exhibited weak to moderate diffused cytoplasmic and granular staining, whereas the holocrine cells exhibited moderate to intense diffused cytoplasmic and granular enzyme activity (figure 5). The granular staining in both the cell types was eserine-resistant. The spermheads in the lumina of the tubules also showed eserine-resistant esterase activity. The staining intensities in both types of cells were slightly reduced during the intervening period (figure 6). During the postbreeding period the principal cells exhibited weak diffused cytoplasmic staining, whereas the staining was intense and granular in the holocrine cells (figure 7). The granular staining in the holocrine cells and the sperm-debris in the lumina of the tubules was eserine-resistant (figure 8).

4. Discussion

Verne and Hebert (1952) reported that the esterase activity appears in the epididymis of the rat earlier than in the testes during the development. Malaty and Bourne (1954) showed that the epididymis of a 12-year boy gives a slight positive esterase activity. Presence of esterase activity has also been reported in the epididymal epithelium of several mammals as described in § 1. The present histochemical results indicate that the epithelial cells in the epididymis of the bat contain non-specific esterases capable of hydrolyzing α NA and 5 BIA.

In recent years, a dual localization of hydrolytic enzymes in the cells has been suggested and established. Several hydrolytic enzymes such as β -glucuronidase, acid phosphatase etc. have been demonstrated in the endoplasmic reticulum and lysosomes. In the present investigation also the enzyme activity was seen as diffused cytoplasmic and granular in nature, the latter being eserine-resistant. The diffused cytoplasmic activity may be correlated with the esterases in the endoplasmic reticulum and the granular activity with the lysosomes. Similar histo-

chemical studies on the epididymis of man (Malaty and Bourne 1954), ram, rabbit, rat and hamster (Moniem and Glover 1972) also revealed diffused cytoplasmic and granular esterase activities.

The present results indicate that the epididymal esterases in bat undergo cyclic variations according to the sex-cycles. During sexual quiescence the diffused cytoplasmic esterase activity is weak in the epithelial cells, both diffused cytoplasmic and granular activities gradually increase from prebreeding period and become intense during the first active breeding period. With slight reduction in the staining during the intervening period, again, the enzyme activities increase during the second active breeding period. During the postbreeding period of regression the diffused cytoplasmic activity decreases but the granular (lysosomal) activity remains unchanged. The bioassay studies on total esterase activity and eserine-resistant esterase activity in the epididymis of this bat (Mote 1981) also substantiate the cyclic variations.

These results indirectly indicate that the epididymal esterases are dependent on the testicular hormones. This conclusion is based on some circumstantial evidences involving castration and hormone replacement studies. Allen and Slater (1957) observed no change in the aliesterase activity in ciliated cells of lobes 2, 3 and 7 of epididymis but the esterase activity was depressed in all other cells after castration. They further reported that testosterone propionate reversed the effects of castration. Hunter and Allen (1959) and Allen and Hunter (1960) obtained seven bands with esterase activity from mouse epididymis by starch-gel electrophoresis. The bands were designated as A to G. Castration was found to depress the activity of A, B and C bands and abolished the activity of D, E and G bands but increased the activity of F band. Androgen administration reversed these effects. These studies indicate that the epididymal esterases are dependent on androgen levels.

Of particular interest is the observation that the holocrine cells are present in the epididymis of this bat. The literature on holocrine cells was reviewed by Martan and Allen (1964). Recently, Vibhute (1981) studied epididymal mucosubstances in eleven species of bats and noted the holocrine cells only in *Rousettus leschenaulti* and *Hipposideros fulvus fulvus*. Vogel (1967) reported that 5 min incubation of rat epididymis with α NA gives an intense tinge in entire epithelium but when the incubation time is reduced to 2 min only holocrine and basal cells show enzyme activity. The present investigation also revealed the presence of non-specific esterases in holocrine cells of the bat epididymis. According to Martan and Allen (1964) acid phosphatase and aliesterase may be the secretory products of holocrine cells. They may be liberated into the lumen of the duct when the cells degenerate and would contribute to the seminal fluid. Such enzymes are known in the seminal fluid and may play a role in the breakdown of certain phosphate esters and lipids prior to their metabolism by spermatozoa.

References

- Allen J M and Hunter R L 1960 A histochemical study of enzymes in the epididymis of normal, castrated and hormone replaced castrated mice separated by zone electrophoresis in starch gels; *J. Histochem. Cytochem.* **8** 50-57

- M and Slater J J 1957 A chemical and histochemical study of alkaline phosphatase and esterase in the epididymis of normal and castrate mice; *Anat. Rec.* **129** 255-273
- 7 G 1976 *Studies on β -glucuronidase of Bat in seasonal Breeding Cycle*; Ph.D. Thesis, Shivaji University, Kolhapur, India
- c S R and Kirkeby S 1978 Histochemical studies on genetical control of hormonal enzyme inducibility in the mouse I. Non-specific esterase activity and regional histology of the epididymis; *J. Anat.* **125** 247-265
- Liberty J, Budd G C and Nelson L 1975 Fine structural localization of cholinesterases in the reproductive tract of male white mouse; *Biol. Reprod.* **13** 397-407
- nn G 1971 Histologische und histochemische untersuchungen zur segmenteinteilung des nebenhodens vom rind vor und nach der geschlechtsreife; *Cytobiologie* **3** 37-69
- ri G 1952 *Microscopic histochemistry, principles and practice* (Chicago: University Press)
- S J 1958 *General cytochemical methods* (ed.) J F Danielli (New York and London: Academic Press) pp 375
- J 1959 Factors governing the validity of staining methods for enzymes and their bearing upon the Gomori acid phosphatase technique; *Exp. Cell Res. (Suppl.)* **7** 1-27
- J and Withers R F J 1958 Studies in enzyme cytochemistry V. An appraisal of indogenous reactions for esterase localization; *Proc. R. Soc. (London)* **B148** 520-531
- r R L and Allen J M 1959 The esterases and phosphatases of the epididymis in normal and castrated and vas efferentectomized mice using the zymogram technique; *J. Histochem. Cytochem.* **7** 307
- by S and Blecher S R 1978a Studies on the oxydizing system in Holt's medium for histochemical demonstration of esterase activity; *Acta Histochem.* **62** 44-56
- by S and Blecher S R 1978b Histochemical studies on genetical control of hormonal enzyme inducibility in the mouse II. Esterase isozymes of the normal epididymis demonstrated by substrate variation and by enzyme inhibitors; *Acta Histochem. Cytochem.* **11** 30-40
- W 1968 Lipofuchsin and lysosomes in the human epididymis, fluorescent microscopy and histochemical investigations; *Histochemie* **16** 236-286
- ty H A and Bourne G H 1954 The distribution of 'simple' esterase in human tissues; *Acta Anat.* **21** 249-258
- an J and Allen J M 1964 Morphological and cytochemical properties of the holocrine cells of the epididymis of the mouse; *J. Histochem. Cytochem.* **12** 628-639
- glia T, Telles M F Jr and Lobao C B A 1970 The male-reproductive system of the common armoset (*Callithrix jacchus*); *Acta Anat.* **76** 594-611
- em K A and Glover T D 1972 Comparative histochemical localization of lysosomal enzymes in mammalian epididymis; *J. Anat.* **111** 437-452
- L T 1981 *Studies on non-specific esterases in gonads and associated reproductive organs of some vertebrates*; Ph.D. Thesis, Shivaji University, Kolhapur, India
- r P B 1976 *Studies on mucosubstances in gonads and associated reproductive organs of Bat*; Ph.D. Thesis, Shivaji University, Kolhapur, India
- krishna P A 1947 Post-partum oestrous in the short-nosed fruit bat, *Cynopterus sphinx*; *phnix*; *Curr. Sci.* **16** 186
- J and Hebert S 1952 L'activite esterassique des cellules interstitielles du Eesticule et de l'epidielyme chez le rat; *Ann. Endocrinol.* **13** 924-930
- ate H G 1981 *Studies on mucosubstances in testes and associated reproductive organs of Chiroptera*; Ph.D. Thesis, Shivaji University, Kolhapur, India
- l J 1967 Zur differenzierung der Esterasen in den epithelzellen des nebenhodens der ratte; *Acta Histochem.* **26** 64-72
- hstein M, Meisel E and Falcon C 1961 Histochemistry of thiolacetic acid esterase: a comparison with non-specific esterase with special regard to the effect of fixatives and inhibitors on localization; *J. Histochem. Cytochem.* **9** 325-329



A study of pupal-adult intermediates produced with juvenoid treatment of *Spodoptera litura* Fabr. pupae

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Abstract. Pupae of *Spodoptera litura* were treated topically with a juvenoid, 6, 7-epoxy-3-ethyl-1 (*p*-ethyl phenoxy)-7 methylnonane. The effects comprised death, production of pupal-adult intermediates of varying grades and adultoids. The production of pupal-adult intermediates was studied in relation to age of the pupa at the time of treatment and the dose of the compound administered. It was found that up to the age of 20 hr the degree of morphogenetic response was directly correlated with the dose of the compound administered and inversely with age, but after this age, an increase in the quantity of the hormone, beyond the effective dose did not further augment the effect.

Keywords. *Spodoptera litura*; juvenoid effect; pupal-adult intermediates.

1. Introduction

Several workers have noted the production of pupal-adult intermediates in endopterygote insects by treating pupae with juvenile hormone or its various analogues (*Sarcophaga bullata*, Srivastava and Gilbert 1969; Bhaskaran 1972; *Tenebrio molitor*, Critchley and Campion 1971; *Trogoderma granarium*, and *Caryedon gonagra*, Metwally and Sehnal 1973; *Trogoderma granarium*, Srivastava and Srivastava 1974; *Ceratitis capitata*, Daoud and Sehnal 1974; *Ephestia kuhniella*, Tan 1975; *Cylas formicarius*, Ram *et al* 1980). In *Sarcophaga bullata*, Srivastava and Gilbert (1969) concluded that the thorax is the first to become refractory to the hormone during pharate adult life, followed by the head and then the abdomen; and that the age of pupa is the most important factor which influences production of intermediates. A dose twice that which is effective in 46 to 50 hr old pupae is ineffective in pupae which are more than 70 hr old. Similarly, Riddiford and Ajami (1973) have observed maximum sensitivity to JH in 24-30 hr old pupae of *Manduca sexta* and greater morphogenetic response with higher doses of the juvenoid treatment and Reddy and Krishnakumaran (1973) noted that in *Tenebrio molitor* highest morphogenetic response is shown by 18-32 hr old pupae. In the same insect, however, Socha and Sehnal (1972) observed more or less uniform morphogenetic response in 0-24 hr old pupae. Further, the intermediates produced with hormone treatment may differ greatly in respect of their characters, possessing the pupal and adult characters in varying proportions. For instance,

Slama *et al* (1974) noted that for the production of adultoids, the quantity of the juvenoid during the sensitive period should either be higher or lower than the critical effective level. They believe that three main parameters determine the morphological and physiological characters of an intermediate: (i) the moment in the sensitive period when the critical effective concentration is traversed, (ii) direction of the concentration change, e.g. whether above or below the critical level and (iii) species specific differences in the sensitive periods between different cells or tissues. The correctness or otherwise of these conditions has not been carefully examined, nor has sufficient attention been paid to the factors which determine the production of intermediates and their different types.

In the present study, different grades of pupal-adult intermediates were produced by treating pupae of *Spodoptera litura* of various ages with different quantities of a juvenoid and an attempt has been made to relate the age and quantity of the compound with the morphogenetic characters of the intermediates.

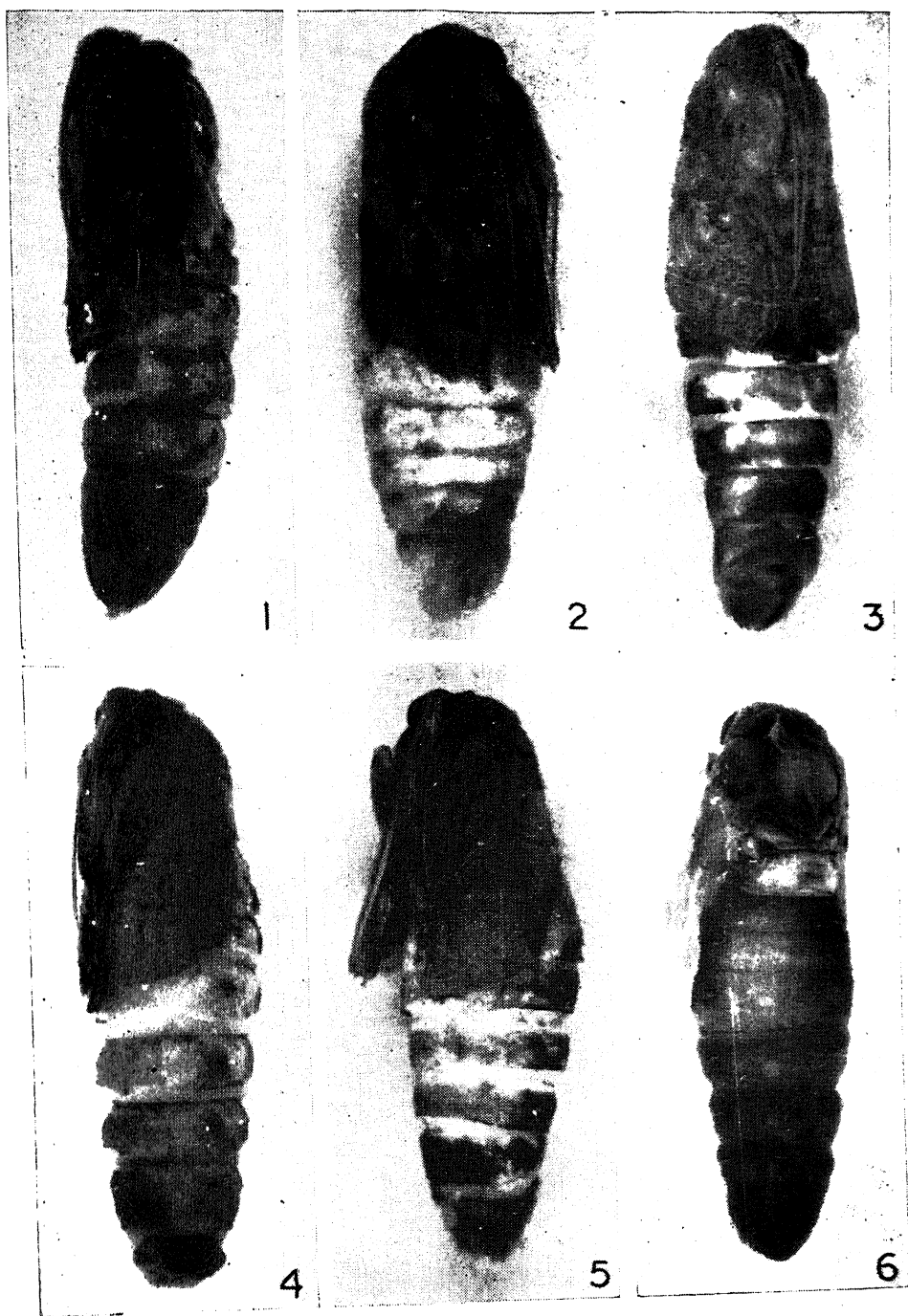
2. Materials and methods

Larvae of tobacco cutworm *Spodoptera litura* were reared on castor leaves in the laboratory at $28 \pm 2^\circ\text{C}$ and allowed to pupate. The pupal life at this temperature was 6–7 days. Pupae of known ages from the stock were placed in cavities made with plasticine and known quantities of the juvenoid, 6, 7-epoxy-3-ethyl-1 (P-ethyl phenoxy)-7 methylnonane *cis/trans* mixture (RO-10-3108/018 of R. Maag Ltd.) dissolved in acetone were topically applied to the anterior abdominal region dorsally with the help of a microapplicator. Controls were similarly treated with acetone only. After emergence of the controls, the old pupal cuticle of the unemerged experimental pupae was carefully removed and the specimens studied for their morphological characters. Specimens which died prior to the completion of developmental period were disregarded in evaluating the results.

3. Results

Pupae of *S. litura* (2, 14, 20, 26 and 38 hr old) were treated with five different quantities, viz. 2, 5, 10, 15 and $25\text{ }\mu\text{g}$ of the juvenoid. The effect comprised death, failure of emergence and emergence of adultoids. When the pupal cuticle of the specimens showing failure of emergence was removed, they were all found to be pupal-adult intermediates of different categories. On the basis of the combinations of pupal and adult characters, these intermediates were classified into the following six grades (table 1 and figures 1–6).

Figures 1–6. Pupal—adult intermediates of varying grades of *S. litura*. 1. Grade I showing adult external structures with bifurcated proboscis. 2. Grade II showing anterior abdomen pupal, wings developed but unstretched and proboscis bifurcated. 3. Grade III showing anterior and middle abdomen pupal, wings developed but unstretched and proboscis bifurcated. 4. Grade IV showing anterior and middle abdomen pupal wings fused and pigmented and proboscis bifurcated. 5. Grade V showing pupal abdomen except tip of abdomen is adult-like, wings non-pigmented and proboscis bifurcated. 6. Grade VI showing whole body pupal.



Figures 1-6.

Table 1. Different characters of p-a intermediates in relation to different grades.

	Grades of p-a intermediates					
	I	II	III	IV	V	VI
Adult abdomen	+					
Part of abdomen adult		+	+			
Adult thorax	+	+	+			
Adult head	+	+	+			
Wings unstretched	+	+	+			
Wings fused and pigmented				+		
Wings non-pigmented/undeveloped					+	+
Proboscis bifurcated	+	+	+	+	+	
♂ ext. gen. deformed; socii, gnathos absent; ovip. fused	+					
♂ ext. gen. less developed and deformed; ovip. fused and reduced		+	+	+		
♂ ext. gen. smaller, less developed and deformed, ovip. absent					+	
Fully pigmented compound eyes	+					
Pigmented and less pigmented eyes		+	+	+	+	
Partly pigmented eyes						+
Anterior abdomen pupal		+	+	+	+	+
Middle abdomen pupal			+	+	+	+
Posterior abdomen pupal						+
Pupal thorax						+
Pupal head						+

Grade I: An adultoid with bifurcated proboscis and unstretched wings; external genitalia developed but deformed. In males, socii and gnathos not developed; in females, substitutional ovipositor fused. Eye pigmentation resembles that of normal compound eye.

Grade II: Anterior abdomen pupal; wings [developed but unstretched; proboscis bifurcated. In external genitalia of males, tegumen, uncus, gnathos and socii not developed while the remaining parts are deformed. Substitutional ovipositor in females fused and reduced. Eyes showing a strip-like heavily pigmented zone towards the proboscis and the remaining weakly pigmented zone. The ratio of pigmented and weakly pigmented zone is about 1:6.

Grade III: Anterior and middle abdomen pupal; wings developed but unstretched; proboscis bifurcated. Condition of external genitalia and eye pigmentation and ratio between heavily pigmented and weakly pigmented zones are similar to that of Grade II.

Grade IV: Anterior and middle abdomen pupal; wings fused and pigmented; proboscis bifurcated. Condition of external genitalia and eye pigmentation and ratio between heavily pigmented and weakly pigmented zones are similar to that of Grade II.

Grade V: Abdomen pupal but the tip of abdomen is adult like; wings non-pigmented; proboscis bifurcated. Male genitalia very small and deformed. Tegumen, uncus, gnathos and socii of males not developed. Substitutional ovipositor in females absent. Eye pigmentation similar to that of the eyes of Grade II, but the pigmented area is relatively larger. Ratio between heavily pigmented and weakly pigmented zones is about 1:2.

Grade VI: Whole body pupal. The eye can be differentiated into a strip-like pigmented zone, about 0.39 mm in width and lying between two unpigmented zones and the unpigmented zone toward the proboscis about 0.26 mm in width. Ratio of heavily pigmented and unpigmented areas is about 1:2.

It would be seen from table 1 that in this scoring system, a higher grade signifies more pupal and less imaginal characters which means that lesser pupal-adult transformation has taken place and *vice versa* a lower grade indicates more imaginal and less pupal characters, showing relatively greater pupal-adult transformation. It was also observed that by and large a particular grade of pupal-adult intermediate was produced by the treatment of pupae at a certain age with a certain amount of the juvenoid (JHA).

3.1. Sensitivity to JHA in relation to age and dose

Table 2 gives the number of pupae of different ages which died, produced p-a intermediates or gave rise to adultoids as a result of treatment of pupae of different ages with different quantities of the juvenoid. It also indicates the grades of the intermediates. It would be clear from the table that lethal action was more pronounced when the treatment was given to early pupa (2 hr old) and as the age of treatment increased, there was a gradual decrease in the number of pupal deaths irrespective of the doses tried. All the treated pupae which failed to emerge were p-a intermediates of varying grades.

Table 2 and figures 7 and 8 give the number and grades of the p-a intermediates produced by the treatment of pupae of different ages with different quantities of the compound. It would be observed that with each of the five doses of the juvenoid tried here, treatment of the youngest pupae (2 hr old) led to maximum morphogenetic response, viz., intermediates produced had maximum pupal characters and as the age of the pupa increased, there was a corresponding decrease in such response and more adult characters were shown by the intermediates produced.

Treatment of 38 hr old pupae produced very low morphogenetic response and adult organs had developed although they were often deformed, e.g., the proboscis was bifurcated and the wings were unstretched (Grade I). In some cases, adultoids with crumpled wings emerged.

Table 2. Treatment of 2-38 hr old pupae of *S. litura* with 2-25 μ g of the JHA.

Age of pupae (in hr)	Dose (in μ g)	No. of pupae treated	No. dead	No. and score of pupal-adult intermediates		No. of adultoids
2	2	25	8	17	V	0
14	2	25	6	19	III	0
20	2	25	2	23	II	0
26	2	25	2	23	II	0
38	2	25	0	21	I	4
2	5	25	11	14	V	0
14	5	25	2	23	IV	0
20	5	25	4	21	III	0
26	5	25	2	23	II	0
38	5	25	0	22	I	3
2	10	25	8	17	VI	0
14	10	25	4	21	IV	0
20	10	25	4	21	III	0
26	10	25	2	23	II	0
38	10	25	2	20	I	3
2	15	25	6	19	VI	0
14	15	25	7	18	IV	0
20	15	25	6	19	IV	0
26	15	25	3	22	II	0
38	15	25	4	18	I	3
2	25	25	7	18	VI	0
14	25	25	3	22	IV	0
20	25	25	3	22	IV	0
26	25	25	3	22	II	0
38	25	25	3	22	I	0

It may also be noted that there was only a slight increase in morphogenetic response by the treatment of 2 hr, 14 hr and 20 hr old pupae with increasing doses of the juvenoid. Treatment of 26 hr and 38 hr old pupae with larger doses of the compound brought no worthwhile difference. A few adultoids emerged with the application of 2-15 μ g to 38 hr old pupa only.

4. Discussion

Williams (1961) described JH as the '*status quo*' hormone and regarded that the classical *status quo* effect on the treatment of larva or pupa with JH is manifested in the form of a supernumerary larval or pupal moult. This would presumably happen when the larval-pupal or pupal-adult transformation is fully arrested by the administration of the hormone before the process of transformation begins and quantity of hormone necessary to bring about the arrest of transformation is available.

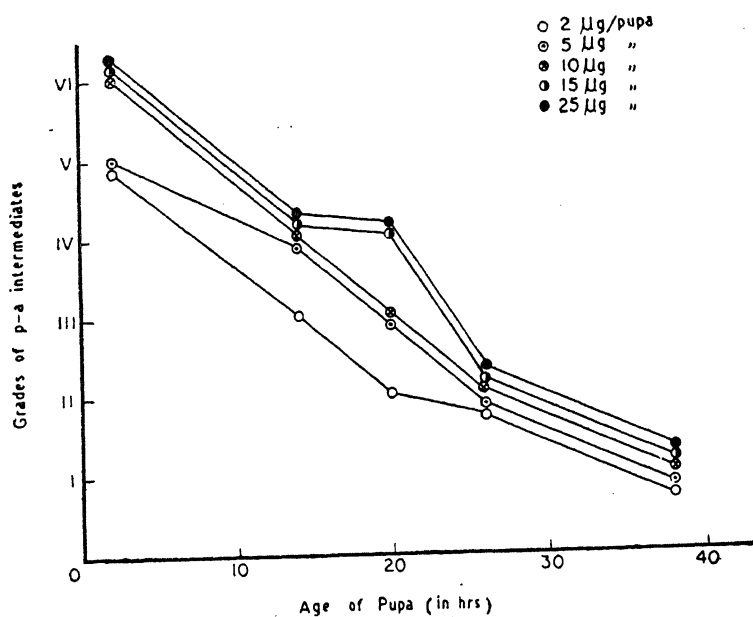


Figure 7. Different grades of p-a intermediates produced by the treatment of pupae of *S. litura* of different ages with JHA.

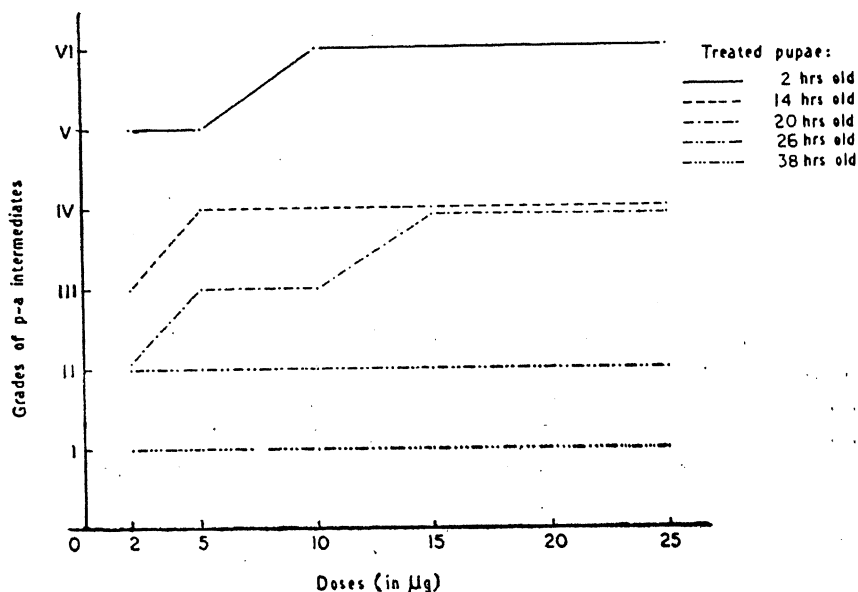


Figure 8. Different grades of p-a intermediates produced by treatment of pupae of *S. litura* with different quantities of JHA.

It is clear from the present observation that the age of the pupa and dose of the juvenoid administered are crucial factors which regulate the occurrence and nature of its morphogenetic response. We shall deal with these two factors separately with reference to the present observations in *Spodoptera litura*.

4.1. Morphogenetic response in relation to age

Several workers have noted that the age of the pupa is a very important factor in the production of p-a intermediates and found that treatment of early pupae with juvenoids is most effective in producing intermediate forms (Srivastava and Gilbert 1969 in *S. bullata*; Critchley and Campion 1971 in *T. molitor*; Metwally and Sehnael 1973 in *T. granarium* and *C. gonagra*; Bagley and Baurnefeind 1972 in *P. interpunctella*; Bhatnagar-Thomas 1972 and Srivastava and Srivastava 1974 in *T. granarium*; Tan 1975 in *E. kuhniella*, Srivastava 1980 in several stored grain insects). In *T. molitor*, Reddy and Krishnakumaran (1973) noted that the highest morphogenetic response is shown when 16-32 hr old pupae are administered the juvenoid, but in the same insect Socha and Sehnael (1972) noted more or less uniform morphogenetic response on treatment of 0-24 hr old pupa. In *Manduca sexta*, Riddiford and Ajami (1973) have observed maximum sensitivity to JH in 24-30 hr old pupa. Recently, in pupae of *Cylas formicarius*, Ram *et al* (1980) have noted juvenilising activity in 61.2% cases when treatment was given to newly moulted pupae and only in 11.5% when 2-day old pupae were similarly treated with the same compound.

On the basis of the morphological characters of different categories of p-a intermediates produced by us in *S. litura*, we find that the age of the pupa is most important with all the doses of the JHA tried. It is clearly established in this insect that the youngest pupa (2 hr old) shows highest morphogenetic response and as the age of treatment of pupa increases there is a gradual decline in the effectivity of the compound.

4.2. Morphogenetic response in relation to dose

Bhatnagar-Thomas (1972) in *T. granarium* observed that treatment of early pupae with very low concentrations of JHA produced no visible morphogenetic effect and normal emergence occurred, whereas with higher concentrations, the number of insects showing abnormalities increased in more or less direct ratio with the concentration of JHA. She also noted that in the case of treatment of late pupae, only higher concentration was effective in producing abnormal forms up to a certain extent. However, Riddiford and Ajami (1973) in *M. sexta* and Ram *et al* (1980) in *C. formicarius* have noted that the quantity of compound administered is also an important factor in producing morphogenetic effect at the time of maximal sensitivity only along with the age of pupa. Slama *et al* (1974) have noted that application of different doses of the juvenoids during the critical period evokes the formation of intermediate forms between the previous and subsequent developmental stages. Our observation on *Spodoptera* pupae have shown that up to 20 hr there is an increase in morphogenetic response with increasing doses of JHA but an increase of JHA dose has no effect when older pupae (26 and 38 hr old) are treated. It has been observed that when 2 hr old pupae were treated with higher doses (10-25 μ g) pupal-adult transformation was more or less completely inhibited and p-a intermediates of grade VI were produced, but with lower doses (2-5 μ g), the differentiation of proboscis, thorax and posterior abdomen was not inhibited although the external genitalia were deformed (Grade V). When 14 hr old pupae were treated with the lowest dose (2 μ g), the development of thorax, wings and

posterior abdomen with comparatively less developed and deformed external genitalia could not be inhibited (Grade III) and with higher doses (5–25 μg), differentiation of thorax and posterior abdomen was not inhibited (Grade IV). Finally when 20 hr old pupae were treated with 2 μg , the differentiation of the middle abdomen also could not be inhibited (Grade II). In short, the twin factors of age and quantity of hormone administered together determine the extent of inhibition of p-a transformation up to the age of about 20 hr but beyond this age an increase in the dose of juvenoid above the effective quantity does not enhance the juvenilising effect.

The production of larval-pupal or pupal-adult intermediates of different grades by JH administration in different quantities and to larvae or pupae of different ages shows that (i) the critical time for the onset of the transformation of the different parts or organs of the body of an insect is different and (ii) similarly the critical concentration of the hormone required to inhibit such transformation is also different. Slama *et al* (1974) and Willis (1974) had also shown earlier that the critical periods for different organs and cells in a larva or pupa occur at different times. In *S. bullata*, Srivastava and Gilbert (1969) noted that the thorax is the first to become refractory to hormone during pharate adult life followed by the head and then the abdomen. Gilbert and Schneiderman (1960) in *A. polyphemus* and Metwally and Sehna1 (1973) in *T. granarium* and *C. gonagra* found that external genitalia and the abdomen as a whole were the first sensitive parts, then the thoracic structures and finally the appendages and mouth parts. In many species of endopterygotes, Slama (1971) had noted that the epidermal cells of the wing lobes, thoracic and head appendages, and external genitalia lose their sensitivity to juvenoids sooner than the epidermal cells of abdomen. In stored grain insects, Srivastava (1980) noted that the order of decreasing sensitivity was abdomen > thorax > head. In *Spodoptera* pupae, the sequence of transformation of different body regions on the basis of different grades of pupal-adult intermediates can be described as follows: anterior abdomen > middle abdomen > posterior abdomen, external genitalia and wings > head and thorax. Bowers and Williams (1964), Krishnakumaran *et al* (1967) and Riddiford and Ajami (1973) believe that in *Manduca sexta*, maximal sensitivity to exogenous JH occurs at the beginning of epidermal retraction which is immediately followed by DNA synthesis, prerequisite to the formation of adult cuticle. Thus, according to this view, in order to be effective an increased titre of JH/JHA should be available in the insect system and it should start action before the process of cellular differentiation is set into motion, probably at a time when the gene sets are being reprogrammed for a new developmental cycle.

Metwally and Sehna1 (1973) have observed that even the most severely affected specimens of *Trogoderma* resulting from pupal treatment, displayed slightly pigmented eyes, outlines of segmentation of appendages, and most important of these, the absence of pupal hair. The maximally affected individuals of *Caryedon* also resembled normal pupae except that their eyes and appendages showed adult-like differentiation. In the course of our observations published elsewhere, we have noted that the pigmentation of the developing compound eyes as seen in these intermediates, is not normal. In fact, the pigmented part comprises an area where an excessive deposition of pigmented material in the cuticle occurs and

in this area or areas, ommatidial differentiation and development are greatly inhibited. Such pigmentation, therefore, does not indicate normal or near normal development of the ommatidia in comparison to the unpigmented region which is assumed to indicate lack of ommatidial development and from a study of the eyes of different grades of p-a intermediates it becomes clear that the extent of abnormal (i.e. heavy) pigmentation and ratio of heavily pigmented and unpigmented/weakly pigmented zones increases, while the ommatidial differentiation decreases with increasing score of p-a intermediates.

The present work indicates that in the case of *S. litura*, the order of critical periods for the metamorphic transformation of several parts or organs can be separately worked out if similar but a more intensive study was carried with the pupal period relatively prolonged, say by rearing the pupae at lower temperature.

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References

- Bagley R W and Bauernfeind J C 1972 in *Insect juvenile Hormones*. (ed) J J Menn and M Beroza (New York : Academic Press)
- Bhaskaran G 1972 Inhibition of imriginal differentiation in *Sarcophaga bullata* by juvenile hormone ; *J. Exp. Zool.* **182** 127-142
- Bhatnagar-Thomas P L 1972 Laboratory evaluation of synthetic juvenile hormone analogue for the control of *Trogoderma granarium* Everts ; *Indian J. Entomol.* **34** 87-93
- Bowers B and Williams C M 1964 Physiology of insect diapause-XII. DNA synthesis during the metamorphosis of *Cecropia* silkworm ; *Biol. Bull. (Woods Hole Mass.)* **126** 205-219
- Critchley B R and Campion D G 1971 Effect of synthetic juvenile hormone and a juvenile hormone analogue, methyl farnesoate dihydrochloride, on pupal development of the yellow mealworm *Tenebrio molitor* L ; *Bull. Entomol. Res.* **61** 293-297
- Daoud D S and Sehnaal F 1974 Effects of juvenoids on the Mediterranean fruit fly, *Ceratitis capitata* (Wied.) (Diptera, Tephritidae) ; *Bull. Entomol. Res.* **64** 643-651
- Gilbert L I and Schneiderman H A 1960 The development of a bioassay for the juvenile hormone of insects ; *Trans. Am. Microsc. Soc.* **79** 38-67
- Krishnakumaran A, Berry S J, Oberlander H and Schneiderman H A 1967 Nucleic acid synthesis during insect development-II. Control of DNA synthesis in the *Cecropia* silkworm and other Saturniid moths ; *J. Insect Physiol.* **13** 1-57
- Metwally M M and Sehnaal F 1973 Effects of juvenile hormone analogues on the metamorphosis of beetles *Trogoderma granarium* and *Caryedon gonagra* ; *Biol. Bull. (Woods Hole Mass.)* **144** 368-382
- Ram G M, Rao B K, Thakur S S and Rao P N 1980 Effectivity of a synthetic juvenile hormone on the pupae of sweet potato weevil, *Cylas formicarius* F. (Coleoptera : Curculionidae) ; *Proc. Indian Acad. Parasitol.* **1** 30-34
- Reddy G and Krishnakumaran A 1973 Changes in the morphogenetic response of *Tenebrio molitor* pupae to juvenile hormone in relation to age ; *J. Insect Physiol.* **19** 773-780
- Riddiford L M and Ajami A M 1973 Juvenile hormone : its assay and effects on pupae of *Manduca sexta* ; *J. Insect Physiol.* **19** 749-762
- Slama K 1971 Insect juvenile hormone analogues ; *Annu. Rev. Biochem.* **49** 1079-1102
- Slama K, Romanuk M and Sorm F 1974 *Insect hormones and bioanalogues* (Wien-New York : Springer Verlag)

- Socha R and Sehna F 1972 Inhibition of adult development in *Tenebrio molitor* by insect hormones and antibiotics; *J. Insect Physiol.* **18** 317-337
- Srivastava R C 1980 *Effect of certain juvenile hormone analogues on the biology of certain stored grain insects*; D.Phil. Thesis, University of Allahabad, Allahabad.
- Srivastava U S and Gilbert L I 1969 The influence of juvenile hormone on the metamorphosis of *Sarcophaga bullata*; *J. Insect Physiol.* **15** 177-189
- Srivastava U S and Srivastava R C 1974 Effects of juvenile hormone analogue on certain aspects of the biology of *Trogoderma granarium* Everts; *Proc. Natl. Acad. Sci.* **44** 99-120
- Tan K H 1975 Effect of a synthetic juvenile hormone and some analogues on *Ephesia* spp. (Lepidoptera-Phycitidae); *Ann. Appl. Biol.* **80** 137-146
- Williams C M 1961 The juvenile hormone II. Its role in the endocrine control of molting, pupation and adult development in the Cecropia silkworm; *Biol. Bull. (Woods Hole Mass.)* **121** 572-585
- Willis J H 1974 Morphogenetic action of insect hormones; *Annu. Rev. Entomol.* **19** 97-115

A comparative study on certain biochemical aspects of red and white myotomal muscles of the black skipjack tuna, *Euthynnus affinis* Cantor

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Abstract. The biochemical assay of certain metabolites of the red and white myotomal muscles of the tuna, *Euthynnus affinis* Cantor has been carried out. The metabolites exhibited a marked variation in their distribution pattern in red and white muscles. The narrow red fibres are characterised by higher levels of lipid, glycogen, myoglobin and SDH while the broader white fibres had lesser amount of the above metabolites. The distribution of metabolites—the myoglobin and SDH, revealed a gradient from the superficial towards the inner layers of the red myotomal muscle in both the pectoral and middle regions. The physiological relevance of these biochemical variations in diverse regions of the red and white muscle is discussed.

Keywords. Skipjack tuna; red and white muscles; protein; fuel reserves; myoglobin; SDH, *Euthynnus affinis*.

1. Introduction

Tuna are actively swimming, commercially important teleosts, exhibiting unique adaptation for maintaining a higher body temperature than the surrounding ambient medium. Similar to certain other teleosts, tuna also possess two types of myotomal muscles—the red and white, with the red muscle lying near the spine and constituting about 5 to 20% of the total body weight (Modigh and Tota 1975). Morphological and biochemical investigations on red and white muscles of fishes have elaborated the functional differences between them (Love 1970). Generally the red fibres are adapted for long-term cruising movements, utilizing lipid as the main source of energy and the white fibres for short-term activity metabolising glycogen as the chief fuel (George 1962; Black *et al* 1962; Bilinisky 1963; Bone 1966; Love 1970). However, relatively very little information is available regarding the physiology of these muscles and their nutritional significance in the black skipjack tuna, *Euthynnus affinis*.

Accordingly a study has been undertaken to elaborate the comparative biochemical aspects of these red and white muscles in *Euthynnus affinis* Cantor.

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2. Materials and methods

Investigations were carried out on tuna weighing 2 to 3 kg having a range of 35 to 55 cm total body length. The fishes were collected from boats immediately on landing at the Shankumugham Beach at Trivandrum and were transported immediately in refrigerated containers to the laboratory. The muscle samples were excised from the superficial, middle and inner most layers from three different regions of the body, viz, the pectoral, middle and the caudal regions (single sample) for biochemical assay by employing standard analytical techniques. The following methods were employed for the estimation of lipid, glycogen, total protein, myoglobin and SDH.

- | | |
|---------------|---|
| (a) Lipid | Nayeemunisa and Rao (1972) |
| (b) Glycogen | Anthrone Reagent technique of Seifter <i>et al</i> (1950) |
| (c) Protein | Wong's microkjeldhal method (1923) |
| (d) Myoglobin | Tappan and Raynaferjee (1957) |
| (e) SDH | Kun and Abood (1949) using the tetrazolium salt as the electron acceptor. |

The optical density of the aliquots obtained was measured in a photoelectric colorimeter (Bausch and Lomb, Spectronic 20).

3. Results

The data on biochemical aspects of these muscles are shown in table 1. Regarding moisture content, the pectoral and middle regions of the red muscle showed only a very slight variation between the superficial layer and the inner layer (Pectoral PS=69.68%; PM=70.71% and PI=70.97% and middle MS=70.09%; MM=69.88% and MI=69.60%) with the caudal region exhibiting a moisture level of 69.84%. The white muscle exhibited relatively higher percentage of moisture 72.57%.

Comparatively, the protein levels of the red muscle did not reveal any variation with the middle layer of the pectoral region having the maximum amount of protein (20.08%). The inner layer of both pectoral and middle regions exhibited slightly lower values (19.23% and 19.65%). The caudal region had 19.91%. The white muscle exhibited a relatively higher protein level (22.48%).

The maximal amount of lipid has been recorded from the middle layers of pectoral and inner layer of middle regions (17.02% and 14.98%). The superficial layers of these regions showed slightly lower values of lipid (15.07% and 13.26%). Interestingly enough both these regions exhibited a gradient in distribution of lipid with the maximum being at the inner and minimum in the superficial. The caudal region had a lower level (12.42%) of lipid. Regarding the white muscle the lipid concentration was very much lower than that of the red muscle (6.96%).

The middle layers of pectoral and middle regions exhibited maximal quanta of glycogen (PM=300.95 and MM=365.85 $\mu\text{g}/100\text{mg}$) with a minimal amount at the inner layer (PI=230.99 and MI=262.63 $\mu\text{g}/100\text{mg}$). The superficial layers showed 233.38 and 284.49 $\mu\text{g}/100\text{mg}$ of glycogen in the pectoral and middle regions respectively. A higher amount of glycogen (323.61 $\mu\text{g}/100\text{mg}$) was

Table 1. Biochemical composition of red and white muscle of *Eutymnus affinis* Cantor.

Body regions	Moisture (% gm wet wt.)	Protein (% gm wet wt.)	Lipid (% dry wt/gm)	Glycogen (μ g/100 mg wet tissue)	Myoglobin (mg/gm wet tissue)	SDH (μ gm formazan min/g)
Pectoral superficial	69.68 \pm (0.32)	19.79 \pm (0.40)	15.07 \pm (0.37)	233.3557 \pm (30.87)	8.6293 \pm (0.30)	42.85 \pm (4.15)
Pectoral middle	70.71 \pm (0.12)	20.08 \pm (0.29)	17.02 \pm (0.56)	300.9526 \pm (30.65)	12.0627 \pm (0.62)	57.46 \pm (6.18)
Pectoral inner	70.97 \pm (0.27)	19.23 \pm (0.76)	16.21 \pm (0.52)	230.9875 \pm (32.25)	13.3008 \pm (1.24)	59.19 \pm (5.65)
Middle superficial	70.09 \pm (0.24)	19.93 \pm (0.42)	13.26 \pm (0.59)	284.9942 \pm (36.54)	8.1042 \pm (1.14)	45.67 \pm (10.54)
Middle middle	69.88 \pm (0.27)	19.50 \pm (0.32)	14.07 \pm (0.68)	365.8492 \pm (41.07)	14.5783 \pm (1.21)	62.49 \pm (8.11)
Middle inner	69.60 \pm (0.27)	19.65 \pm (0.28)	14.98 \pm (0.26)	262.6330 \pm (37.30)	15.8272 \pm (1.33)	65.68 \pm (10.59)
Caudal red	69.84 \pm (0.17)	19.91 \pm (0.23)	12.92 \pm (0.49)	323.6126 \pm (40.17)	13.4902 \pm (1.06)	68.41 \pm (9.38)
White muscle	72.54 \pm (0.27)	22.14 \pm (0.49)	6.96 \pm (1.29)	60.1494 \pm (28.85)	2.4635 \pm (0.16)	27.87 \pm (3.68)

The number of fish examined in each case was 10.
Mean value of observation is given with standard error in brackets.

discernible at the caudal region. As for the white muscle the glycogen concentration was significantly very much lower ($60.15 \mu\text{g}/100 \text{ mg}$).

The myoglobin levels of both pectoral and middle regions exhibited a marked gradient from the superficial to inner layers (PS—8.6293 ; PM—12.0627 ; PI—13.3008 and MS—8.1042 ; MM—14.5783 and MI—15.8273 mg/g wet tissue. The caudal region had 13.4902 mg/gm of myoglobin. Relatively, the white muscle revealed only a very much lower value for myoglobin (2.4635 mg/g).

The concentration of SDH at the pectoral and middle regions exhibited an increasing gradient from superficial towards the inner layers—viz., (Pectoral ; PS—42.85 ; PM—57.46 ; and PI—59.19 and middle ; MS—45.67 ; MM—62.49 and MI—65.68). The SDH level in the caudal region was relatively higher (68.41) while the concentration in the white muscle was very much lower ($27.87 \mu\text{gm—formazan/min/g}$).

4. Discussion

The moisture concentration revealed only a very narrow range of variation in the red muscle samples from different regions of the tuna fish. Nevertheless a comparatively higher percentage has been noted in the white muscle; Alexander (1955), Love (1970) and Chinnamma (1975) had reported a higher value of moisture content in white muscles of fishes.

Studies on the protein content of *Euthynnus affinis* did not reveal any significant variation in the red muscle samples. However, protein level was comparatively higher in the white muscle. This is similar to those of the avian muscles such as pigeon pectoralis muscle as reported by Pishawikar (1961). It has been suggested that the higher total protein content in the white muscle is due to structural proteins, such as actin and myosin and the higher water soluble protein in the red fibers is due to their higher enzyme concentration (George and Berger in *Avian myology* 1966). Further, lower levels of total protein had been reported in the dark muscles of *Sardinia melanosticta* (Fujikawa and Naganuma 1936) ; *Scomber scombrus* and *Thunnus thynnus* (Braekkan 1959) which are also in conformity with the values observed in the dark muscles of *Euthynnus affinis*.

Regarding fuel reserves, data indicate that the red muscle fibres have a higher fuel reserve with a preferential dependence on lipid. Lipid constitutes an important source of fuel reserve for muscle contraction ; the metabolism of which yield sufficient ATP (West *et al* 1956). In tuna, the relatively higher level of lipid in the pectoral region may be due to the continuous activity of the pectoral fins. Further, it is also possible that the red muscle fibres are capable of providing the requisite amount of energy for the slow and sustained contraction for the long term swimming activity of the fish by the aerobic oxidation of the lipids. The relatively lower level of lipid in the white muscle may be due to their non-involvement in slow and sustained activity and are mainly meant for fast spurts of movement using mainly glycogen as the fuel. The breakdown of lipid is most evident in those fish which migrate without feeding (Bilinisky 1963). It has been reported that in the trout (*Salmo gairdnerii*) the ability of dark muscles to oxidise fatty acid was much greater than that of the ordinary muscles by the presence of an enzyme system in the red muscle (Bilinisky 1963). The observations of Drummond

and Black (1960) had revealed that fat metabolism provides the energy for sustained swimming in the up stream migration of salmonids. Further, studies of Braekkan (1959) in *Clupea harengus*; *Gadus virens*, *Salmo salar*; Alexander (1955) in *Scatophagus argus* and *Labeo rohita*; George (1962) in *Rastrelliger kanagurta*; Zama (1963) in *Thynnus orientalis* etc., had revealed a much higher lipid concentration in the red muscles of these fish.

Glycogen is one of the major fuel reserves of the muscle. Studies on *Euthynnus affinis* have revealed a higher concentration of glycogen in the red muscle almost ranging over two to three times than that of white muscle. In fishes usually the white muscle produces much of the energy for sudden bursts of activity by anaerobic metabolism (Rayner and Keenan 1967). Apart from this, certain functions have been attributed to the red muscles in fish myotome. Among these, Arloing and Lavocat (1875) have suggested that the two types of fibres—the red and white were active during different phases of swimming. George (1962) had reported in the teleost, *Rastrelliger kanagurta*, the red muscle was adapted for continuous and slow contractions while the white fibres effecting quick and fast contraction. Further, the observations of George (1962); George and Bokdawala (1964); Bone (1966); Love (1970) corroborate the view that the red muscle facilitates continuous muscular activity of the animal.

Comparatively a lower concentration of glycogen was recorded in the tuna white muscle. Bokdawala and George (1967) had suggested that the probable depletion of glycogen in the white muscle may be due to the fact that it might have been used up since the white muscle fibres are involved in quick and sudden movement during capture by utilizing the energy derived from the breakdown of glycogen. Studies by Driedzic and Hochachka (1976) in carp muscle had revealed an increase in glycolytic intermediates during activity. Thus in tuna, *Euthynnus affinis*, the comparatively higher levels of lipid and glycogen in the red muscle indicates the continuous and higher rate of utilization of these fuels for the active swimming habits of this teleost.

The characteristic red colour of the tuna red muscle is due to the preponderance of myoglobin. It plays a salient role in the transport and storage of oxygen in the muscle (Lawrie 1952) and has the capacity for rapid oxygenation and deoxygenation. Thus in tuna, the higher myoglobin levels in the red muscle facilitate a better diffusion of oxygen into the red muscle and function as a store house of oxygen for the aerobic oxidation. Further, in red muscle the main energy source is lipid and it can be metabolised aerobically which warrants a sufficient supply of oxygen. Observations of Modigh and Tota (1975) in *Thunnus thynnus* revealed that mitochondria from deep red muscle consume more than thrice as much oxygen as those from white muscle when the complete electron transport chain is in operation. Moreover, in *Euthynnus affinis*, the higher level of myoglobin in the inner layers of pectoral and middle red muscle regions, wherein the arteries and veins are highly concentrated may possibly have specific role in the production and maintenance of slightly higher body temperature together with the "rete mirabili", which plays a prominent role in these parts of the muscle (Carey 1973).

The data obtained on SDH (succinic dehydrogenase), the prime mover of oxidation in the metabolic process going on in a muscle, indicate that its levels are much higher in the red muscle with an increasing gradient from the superficial to

the inner layers in both pectoral as well as the middle regions. However, the white muscle exhibited a significantly lower SDH level. It is well-known that the level of SDH in different layers of the muscle can be correlated to their oxidative capacity. Further, it provides an indication of the mitochondrial intensity. Hence the higher levels of SDH in the red muscle fibres of the tuna fish reflect its higher oxidative capacity. Similar data have been reported by Talesara and Narang (1979) in mammalian and avian muscles. In fact the relatively higher metabolic demands of red muscle warrant a higher SDH concentration in correlation with the increased myoglobin content.

In consensus, the significant variation discernible in the biochemical parameters, especially fuel reserves, myoglobin and SDH in red muscles are in accord with the specific functional requirements of these red muscles viz., the sustained muscular activity, production of increased metabolic energy for maintaining a higher body temperature than the ambient medium.

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References

- Alexander K M 1955 A comparison of the gross chemical composition of red and white muscles in the two fishes *Scatophagus argus* and *Labeo rohita*; *J. Anim. Morphol. Physiol.* **1** 58-61
- Arloing S and Lavocat A 1875 Recherches Sur l'Anatomie et la physiologie des muscles stress pales et foncees; *Mem. Acad. Sci. Inscript. Toulouse T.* **7** 177-197
- Blinisky E 1963 Utilization of lipid by fish. 1. Fatty acid oxidation by tissue slices from dark and white muscles of Rainbow trout *Salmo gairdneri*; *Can. J. Biochem. Physiol.* **41** 107-112
- Black E C, Connor A R, Lam K C and Chia W G 1962 Changes in glycogen, pyruvate and lactate in rainbow trout (*Salmo gairdneri*) during and following muscular activity; *J. Fish. Res. Board Can.* **19** 409-436
- Bokdawala F D and George J C 1967 A quantitative study of fat, glycogen, lipase and succinic dehydrogenase in fish muscle; *J. Anim. Morphol. Physiol.* **14** 223-230
- Bone Q 1966 On the function of the two types of myotomal muscle fibre in elasmobranch fish; *J. Mor. Biol. Ass. U.K.* **46** 321-349
- Braekkan O R 1956 Function of red muscle in fish; *Nature (London)* **178** 747-748
- Braekkan O R 1959 A comparative study of vitamin in the trunk muscles of fishes; *Fisk. Dir. Skr. Ser. Teknol. Und. Kol.* **3** 42
- Carey F G 1973 Fishes with warm bodies; *Sci. Am.* **228** 36-50
- Chinnamma George 1975 Biochemical differences between the red and white meat of tuna (*Katsuwonus pelamis*) and changes in quality during freezing and storage; *Fish. Technol.* **12** 70-74
- Driedziec W R and Hochachka P W 1976 Control of energy metabolism in carp white muscle; *Am. J. Physiol.* **230** 579-582
- Drummond G I and Black E C 1960 Comparative physiology fuel of muscle contraction; *Annu. Rev. Physiol.* **22** 169-190
- Fujikawa K and Naganuma H 1936 Chemical composition of sardine, *Sardinia melanosticta* (C and V) from Tyosen. 1. Comparative study on dark muscle and white muscle; *Bull. Jpn. Soc Sci. Fish.* **5** 95-102
- George J C 1962 A histophysiological study of the red and white muscles of the mackerel; *Am. Midl. Nat.* **68** 487-494

- George J C and Berger A C 1966 *Avian myology* (New York : Academic Press)
- George J C and Bokdawala F D 1964 Cellular organization and fat utilization in fish muscle ; *J. Anim. Morphol. Physiol.* **11** 124-132
- Kun E and Abood L G 1949 Colorimetric estimation of succinic dehydrogenase by Triphenyl tetrazolium chloride ; *Science* **109** 144-146
- Lawrie R A 1952 Biochemical difference between red and white muscle ; *Nature* **170** 122
- Love R M 1970 *The chemical biology of fishes* (London and New York : Academic Press)
- Modigh M and Tota B 1975 Mitochondrial respiration in the ventricular myocardium and in the white and deep red myotomal muscles of the juvenile tuna fish (*Thunnus thynnus* L.) ; *Acta Physiol. Scand.* **93** 289-294
- Nayeemunisa and Rao K P 1972 Effects of thermal acclimation in the lipid metabolism in the earthworm ; *Lampito mauritii*—A method for lipid estimation ; *Comp. Biochem. Physiol.* **B42** 167-173
- Pishawikar S D 1961 *A study of the phosphates, phosphatases and certain inorganic ions in the pectoralis muscle of some birds with special reference to that of the pigeon*. Doctoral Thesis M.S. University of Baroda, Baroda, India
- Rayner M D and Keenan M J 1967 Role of red and white muscles in the swimming of skip-jack tuna ; *Nature (London)* **214** 392-393
- Seifter S, Dayton S, Novic B and Muntwyler E 1950 The estimation of glycogen with the Anthrone reagent ; *Arch. Biochem.* **25** 191-200
- Talesara C L and Vasdev Narang 1979 A comparative study of myofibrillar ATPase (m-ATPase) and succinic dehydrogenase (SDH) activities in certain specialised muscles of rat (*Rattus norvegicus*) from various representative regions ; *Indian J. Exp. Biol.* **17** 219-221
- Tappan DU and Raynaferjee B 1957 Tissue pigment manifestations of adaptation to high altitudes ; *Am. J. Physiol.* **190** 99-103
- West E S, Todd W R, Manson H S and Bruggen J T V 1956 *A text book of Biochemistry*. (New York: Macmillan Co.)
- Wong 1923 Estimation of proteins in blood plasma ; *J. Biol. Chem.* **55** 427
- Zama K 1963 Studies on the phospholipids of aquatic animals ; *Mem. Fac. Fish. Hokkaido Univ.* **11** pp 73

Circadian basis for the photoperiodic response in the male blackheaded bunting (*Emberiza melanocephala*)

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Abstract. Short day (6 hr light in a 24 hr cycle (LD 6:18)) inhibits growth and development of the testes in male blackheaded buntings, whereas the same (6 hr) nonstimulatory photoperiods in a 36 hr cycle (LD 6:30) induce complete testicular recrudescence and development. In another experiment of 24 hr cycles, using the same (6 hr) main photoperiod, testes were stimulated when the dark period was interrupted by light at 12 to 13 hr after the onset of basic photoperiod (LDLD 6:6:1:11). The results appear to conform to the tenets of the external coincidence model.

Keywords. Blackheaded bunting ; photoperiod ; circadian ; rhythm ; light : dark cycle ; external coincidence model.

1. Introduction

Since the pioneer studies of Hamner (1963) on house finches (*Carpodacus mexicanus*), the nature of the photoperiodic response mechanism(s) has been experimentally investigated in many photoperiodic birds (see reviews, Farner and Lewis 1971 ; Follett 1973 ; Farner 1975 ; Farner *et al* 1977 ; Turek 1978). The results from these experiments agree with the classical Bünning hypothesis which mentions an endogenous circadian rhythm of sensitivity to light as the physiological basis for photoperiodism (Bünning 1973). The validity of a circadian basis for photoperiodic time measurement in birds is generally tested by resonance and night-interruption experiments (see reviews, Follett 1973 ; Farner 1975 ; Turek 1978). Here, we report the results of night-interruption experiments designed to test the influence of an endogenous circadian rhythm in the photoperiodic time measurement of blackheaded buntings.

2. Materials and methods

Wild adult male blackheaded buntings (*Emberiza melanocephala*) were acclimatized to laboratory conditions for a fortnight. These acclimated birds were pretreated

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for 8 weeks with short days (LD 8 : 16) ensuring that they were photosensitive at the time of exposure to different light regimes. Three groups (numbered I, II and III) of birds then were marked individually and held under different programmed photoperiods (LD 6 : 18, LD 6 : 30 and LDLD 6 : 6 : 1 : 11, respectively) for a fixed period (see table 1) inside light-boxes. Food and water were freely available. The birds were lit by fluorescent tubes at an intensity of about 400 lux at perch level. The first experimental photophase was in phase with the pretreatment schedule and commenced at 06.00 hr. The birds were laparotomized at the beginning and end of experiments, and only during the main light phase of the cycle. Testicular growth was assessed as combined testicular weight *in situ* and by comparing with the standard set of gonads of known weights. The error by this method is about (\pm) 20%. The data from one bird of group III that died during the course of study were not included in our statistical analysis. The data were analysed using student's 't' test.

3. Results and discussion

The results are presented in table 1. The birds either of group I (LD 6 : 18) or of II (LD 6 : 30) received equal photoperiods (6 hr) per cycle but only the birds of the latter group responded. Since the extended period of darkness could appear to initiate the gonadal recrudescence, in a separate experiment buntings were held in constant darkness (DD) for 100 days and found not to respond (unpublished results). Further, the birds of group III (LDLD 6 : 6 : 1 : 11) also responded although the total amount of darkness which these birds received per cycle (17 hr) was even less than the amount which birds of group I were experiencing (18 hr). The duration of light also cannot be a factor in initiation of the testicular growth in the buntings, since the total amount of light per cycle given in all the experiments (6 hr or 7 hr) was much shorter than the photoperiodic threshold for the species which lies at 11 to 12 hr light per day (Kumar and Tewary 1982). Further, it is to be noted that a light regimen consisting of 8 hr photoperiod (LD 8 : 16)

Table 1. The gonadal responses of *Emberiza melanocephala* exposed to 3 different light regimes.

Group	Light regime (light : dark)	Light cycle (in hr)	Days of treatment (in weeks)	Combined testicular weight (mg)	
				Initial	Final
I	LD 6 : 18	24	5	9.5 \pm 1.68 (6)	7.00 \pm 0.63 (6)
II	LD 6 : 30	36	5	8.0 \pm 0.00 (7)	285.71 \pm 21.03 (7)
III	LDLD 6 : 6 : 1 : 11	24	6	7.5 \pm 0.50 (6)	216.00 \pm 22.49 (5)

Value in parenthesis gives the number of individuals

for 6 months could not induce the testes of blackheaded buntings (Tewary and Kumar 1982).

It appears that neither the amount of light or dark nor the ratio of light to dark is the determining factor in stimulating the gonadal growth and development in blackheaded buntings. Our data agree with those obtained with similar experiments on other known photoperiodic birds (Hamner 1963, 1964; Follett 1973; Farner 1975; Tewary and Kumar 1981a, b; Chandola *et al* 1976). Such results may best be interpreted on the basis of an endogenous circadian rhythm involvement in the 'photosensitivity' of the hypothalamo/hypophyseal/gonadal system (Follett 1973; Farner 1975; Turek 1978). According to the external coincidence model, first developed by Bünning (1973), a photoperiodic induction occurs if and only if photophase coincides (repeatedly, daily or otherwise) with the photosensitive phase (= photoinducible phase, subjective night) of the circadian rhythm. In the present experiments presumably the birds of group II received 6 hr light at alternate cycles, and of group III were receiving 1 hr light period daily in the photosensitive phase and a response was obtained in both the groups. In contrast, the birds of group I were receiving light periods (6 hr) daily only in the photoinensitive phase (= non-photoinducible phase, subjective day), and hence photostimulation failed to occur.

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References

- Bünning E 1973 *The physiological clock*. 3rd (ed.) (New York, Heidelberg, Berlin: Springer-Verlag)
- Chandola A, Singh R and Thapliyal J P 1976 Evidence for a circadian oscillation in the gonadal response of the tropical weaver bird (*Ploceus philippinus*) to programmed photoperiods; *Chronobiologia* 3 219-227
- Farner D S 1975 Photoperiodic controls in the secretion of gonadotropins in birds; *Am. Zool.* 15 117-135
- Farner D S, Donham R S, Lewis R A, Mattocks P W, Darden T R and Smith J P 1977 The circadian component in the photoperiodic mechanism of the house sparrow, *Passer domesticus*; *Physiol. Zool.* 50 247-268
- Farner D S and Lewis R A 1971 Photoperiodism and reproductive cycles in birds; in *Photophysiology* (ed.), A. C. Giese (New York and London: Academic Press) Vol. 6 pp 325-370
- Follett B K 1973 Circadian rhythms and photoperiodic time measurement in birds; *J. Reprod. Fertil.* 19 5-18
- Hamner W M 1963 Diurnal rhythm and photoperiodism in testicular recrudescence of the house finch; *Science* 142 1294-1295
- Hamner W M 1964 Circadian control of photoperiodism in the house finch demonstrated by interrupted-night experiments; *Nature (London)* 203 1400-1401
- Kumar V and Tewary P D 1982 Response to experimental photoperiods by a migratory bunting, *Emberiza melanocephala*; Ibis (accepted).

- Tewary P D and Kumar 1981a Involvement of circadian rhythm in photoperiodic response in the male common Indian rosefinch *Carpodacus erythrurus*; *Indian J. Exp. Biol.* **19** 77-79
- Tewary P D and Kumar V 1981b Circadian periodicity and the initiation of gonadal growth in the male blackheaded bunting (*Emberiza melanocephala*); *J. Comp. Physiol.* **144** 201-203
- Tewary P D and Kumar V 1982 Photoperiodic response of a subtropical migratory finch, the blackheaded bunting (*Emberiza melanocephala*); *Condor* **84** 168-171
- Turek F W 1978 Diurnal rhythms and the seasonal reproductive cycle in birds; In *Environmental endocrinology*; (ed.) I. Assenmacher and D S Farner (Berlin, Heidelberg, New York: Springer-Verlag) pp 144-152

Steroid metabolism in target related to nuptial plumage production in the Baya weaver bird

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Abstract. To elucidate the possible utilization of gonadal sex hormones by the nuptial plumage producing skin, histochemical localization of 3β -, 3α - and 17β -hydroxysteroid dehydrogenases was carried out in the skin from crown region (characterized by bright yellow plumage) and ventrum, and testes of the Baya weaver bird *Ploceus philippinus* (L) during the breeding phase. Results indicate higher activity of 17β -hydroxysteroid dehydrogenase in the crown skin, when testosterone was used as substrate. Possibly, skin from the crown region actively metabolizes androgens and this in turn is correlated to the production of nuptial plumage.

Keywords. Baya weaver bird ; histochemistry ; steroid dehydrogenases ; skin.

1. Introduction

It is a generally accepted concept that the accessory sex organs and secondary sexual characters of vertebrates are under the control of sex hormones. Sexual dimorphism of birds could be genetically determined (as in house sparrow) or hormone mediated. As in human skin cytosol (Mowszowicz *et al* 1981), androgen receptors have been reported in the uropygial glands of male ducks (Daniel *et al* 1977) which would be quite typical of an androgen target site. Interestingly, an extragonadal direct effect of luteinizing hormone (LH) on the seasonal plumage changes has been proposed in the orange weaver finch *Euplectes franciscanus* (Witchi 1950) and Indian weaver bird *Ploceus philippinus* (L) (Thapliyal and Tewary 1961, 1963 ; Thapliyal and Saxena 1961). It would be noteworthy to find out the advantage of gonadotrophic control over gonadal steroids in the formation of nuptial plumage in the weaver birds. The present work was aimed at finding out whether the crown skin producing bright yellow nuptial plumage of the Baya weaver bird is capable of utilizing gonadal hormones.

2. Material and methods

Adult male Baya weaver birds *Ploceus philippinus* (L), were shot down in Vidyanagar University Campus during their breeding phase (August/September). Part of the defeathered skin from the crown region and ventrum (ventral normal colour skin) was fixed on the AO cryostat chuck maintained at -20°C . Testes of the same birds were also fixed in the cryostat. Sections ($12\text{ }\mu\text{m}$ thick) were cut on the microtome and sections were processed for the demonstration of 3β -hydroxysteroid-dehydrogenase (3β -HSDH; Wattenberg 1958); 3α -hydroxysteroid-dehydrogenase (3α -HSDH; Balough 1966) and 17β -hydroxysteroid-dehydrogenase (17β -HSDH; Kellogg and Glenner 1960). The pH for 3α -HSDH incubation medium was maintained at 7.7 (Ambadkar and Kotak 1978). The control sections in all cases were incubated in media devoid of hormones.

3. Results and discussion

The enzyme intensities have been graded as under :

— Nil, + minimum, ++ moderate, +++ maximum.

In all the three enzymes studied, epidermis revealed more activity than the dermis, both in crown as well in ventrum.

3.1. 3β -HSDH (*pregnenolone*)

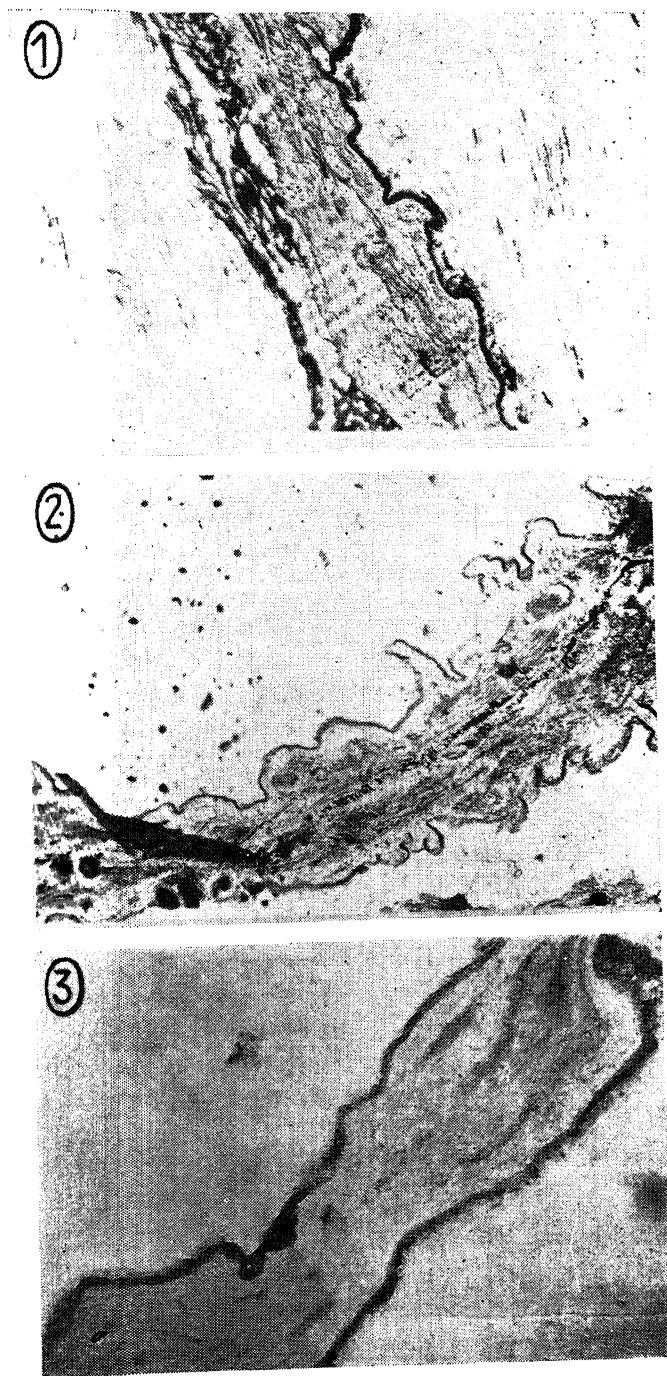
Activity in the ventrum was nil (—, figure 5) and in the crown skin, moderate (++, figure 2). Probably, interconversions involving proandrogens may occur in the crown skin. Testes exhibited high intensity (+++, figure 6) being more or less uniform in the Leydig cells and the seminiferous epithelium. Possibly, the Sertoli cells play an important role in androgen synthesis as the interstitial cells. It is now widely accepted that the Sertoli cells produce steroids that may influence spermatogenesis (Bentley 1976). This aspect in the Baya weaver bird, however, would demand more extensive seasonal investigations, particularly prior to, during and post-reproductive phases *vis-a-vis* lipid cycle in the testicular compartments.

3.2. 3α -HSDH (*androsterone*)

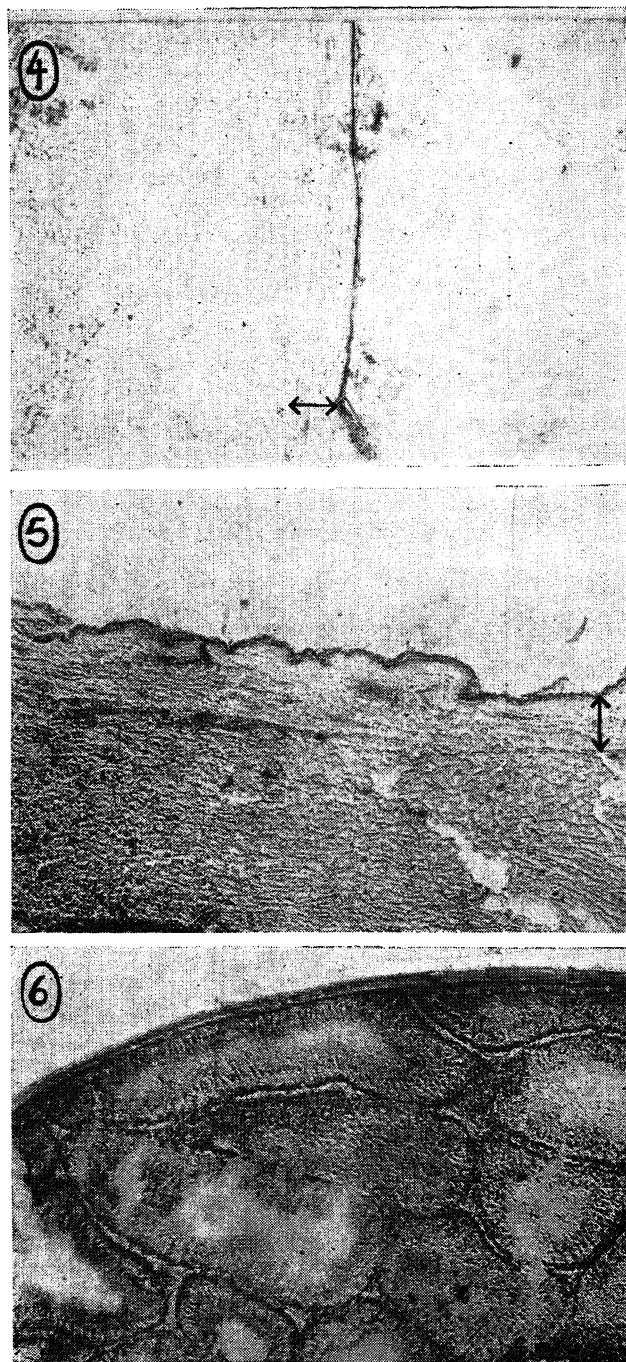
Pattern of localization was more or less same as in case of 17β -HSDH (testosterone) but the intensity was weak (+, figure 3). To a lesser extent, interconversions between Δ^5 -3 hydroxysteroids and Δ^4 -3-ketosteroids may occur in the crown skin (e.g. androsterone to testosterone). Testes and ventrum showed practically no activity.

3.3. 17β -HSDH (*testosterone and estradiol*)

Both, the ventrum and crown skin, showed minimum (+) activity with estradiol as substrate. The female sex hormone does not seem to be metabolized by the integumentary regions. As against this, the enzyme localization with testosterone



Figures 1-3. Sections of Baya skin from crown region ($\times 50$). 1. 17β -HSDH (testosterone) shows intense reaction in the epidermis. 2. Moderate 3β -HSDH (pregnenolone) activity. 3. 3α -HSDH (androsterone) appears lesser.



Figures 4-6. 4 and 5. Ventrum (\longleftrightarrow , rest is pith tissue) reveals feeble 17β -HSDH (testosterone) and 3β -HSDH (pregnenolone) localization ($\times 100$). 6. T.S. of testis shows high 3β -HSDH (pregnenolone) in the seminiferous epithelium ($\times 50$).

revealed moderate to high activity (+ +, + + +) in the crown skin (figure 1) and minimum (+) in the ventrum (figure 4). There is thus good likelihood of 17-OH-steroid to 17-ketosteroid (androgens) turnover in the skin from the crown region (e.g., testosterone to androstenedione or *vice versa*), whereas the ventrum appears to be hypo-sensitive in this regard. Testes exhibited moderate enzyme localization, once again, the seminiferous epithelium and interstitial cells displayed no visible distinction at this time of the year.

In the South African weaver finches, the yellow and black breeding plumage is believed to be under the control of LH (and not androgens) since castration has no effect on plumage change. Also, administration of anterior pituitary extracts to females or non-breeding males, castrate or intact, is followed by the appearance of dark feathers (cited from Turner and Bagnara 1976). However, Ralph *et al* (1965) have demonstrated that there is no direct action of LH on changes in feather colour in these birds. Besides, in castrated birds, augmented output of adrenocortico androgens cannot be ruled out. That avian skin is capable of sex steroid hormone interconversions can be seen from the study on Sebright cocks (George *et al* 1981). These workers have suggested conversion of testosterone to estradiol by skin which is responsible for the typical female feathering trait of males. Other target sites too possess such property; in drakes for example, testosterone is rapidly converted to dihydrotestosterone (DHT) in target organs (Horst and Paulke 1977). Our present report does not permit a conclusive comment on the involvement of androgens in nuptial plumage production. However, it is apparent that the target skin (nuptial plumage producing crown region) is endowed with relatively greater ability for steroid hormone metabolism than the non-target areas.

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References

- Ambadkar P M and Kotak V C 1978 Histochemical observations on 3 α - and 17 β -hydroxysteroid dehydrogenases in extra gonadal tissues of feral blue rock pigeon *Columba livia* G. ; *Indian J. Exp. Biol.* **16** 298-301
- Balough K 1966 Histochemical demonstration on 3 α -hydroxysteroid dehydrogenase activity; *J. Histochem. Cytochem.* **14** 77-83
- Bentley P J 1976 *Hormones in reproduction in Comparative vertebrate endocrinology* (New York : Cambridge University Press) pp 332
- Daniel J V, Vignon F, Assenmacher I and Rochefart H 1977 *Steroids* **30** 703
- George F W, Noble J F and Wilson J D 1981 Female feathering in Sebright cocks is due to conversion of testosterone to estradiol in skin ; *Science* **213** 557-595
- Horst H J and Paulke E 1977 Comparative study of androgen uptake and metabolism in domestic and wild mallard drakes (*Anas platyrhynchos* L.) ; *Gen. Comp. Endocrinol.* **32** 138-145
- Kellogg D A and Glenner G G 1960 Histochemical localization of human term placental 17 β -Estradiol dehydrogenase reaction ; *Nature (London)* **187** 763

- Mowszowicz I, Riahi M, Wright F, Bouchard P, Kuttann F and Mauvais-Jarvis P 1981 Androgen receptor in human skin cytosol ; *J. Clin. Endocrinol. Metab.* **52** 338-344
- Ralph C L, Hall P F and Grinwich D L 1965 Failure to demonstrate a direct action of luteinizing hormone (LH or ICSH) on regenerating feathers in African weaver birds ; *Am. Zool.* **5** 212
- Thapliyal J P and Saxena R N 1961 *Naturwissenschaften* **24** 751-742
- Thapliyal J P and Tewary P D 1961 *Science* **134** 738 cited from : *Reproduction in Indian birds* by J P Thapliyal in Pavo 1978 **16** 151-161
- Thapliyal J P and Tewary P D 1963 *Naturwissenschaften* **15** 529-531
- Wattenberg L W 1958 Microscopic histochemical demonstration of 3 β -ol-dehydrogenase reaction ; *J. Histochem. Cytochem.* **6** 225-232
- Witschi E 1950 Zur biologischen charakterisierung der gonadotropen hormone ; *Naturwissenschaften* **37** 81

Sex pheromone in a stomatopod crustacean *Squilla holoschista*

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Abstract. The stomatopods are well-known for their aggressive and agonistic encounters. The males are normally aggressive ; the females too in the non-reproductive condition show such a behaviour with males. In *S. holoschista* mating is frequent as well as repetitive. The present paper explains whether there is any involvement of sex pheromone. The sex pheromones are considered to be present in ovaries, cement glands as well as oviducal extractions. These substances were tested for their pheromonal activity. The results indicate that there may not be such attraction as evidenced by the lack of mating gestures from the isolated males in the presence of these substances. It is therefore suggested that the mating in the stomatopod, *S. holoschista* is indiscriminate. The physiological effect of such a repeated and indiscriminate mating on the female is discussed.

Keywords. Pheromone ; natural sex attractants ; *Squilla* ; premating gestures.

1. Introduction

The accumulation of evidences drawn from insects led to the introduction of pheromone concept (Karlson and Lüscher 1959). Recently sex attractants in the form of pheromones have been found to exist in several crustaceans (Dahl 1975). However due to lack of proper controlling methods, the mere existence of pheromones in Crustacea is questioned (Dunham 1978).

In decapod crustaceans the behavioural movements may be due to chemical or visual stimuli (Salmon 1965, 1971 ; McLeese 1971 ; Ryan 1966 ; Teytaud 1971). Initial behavioural contact between male and female is followed by mutual exchanges of communicating signals. This helps in transmitting information of species, identification of sex and reproductive drive from one animal to another (Hazlett 1975). Alteration in the agonistic behaviour has been shown to result in the pair of reproductive male and female (Hazlett and Winn 1962 ; Nolan and Salmon 1970). The act of copulation in marine Crustacea varies from one species to another. In general, mating occurs in the freshly moult condition. But there are exceptions to this rule (Hartnoll 1969). In lobsters and anomuran species mating occurs normally between a fresh moult female and an intermoult male (Berry 1970 ; Hazlett 1970, 1972). In some hermit crabs, Hazlett (1972)

observed frequent mating and copulation was prolonged in hard-shelled crabs. Dingle and Caldwell (1972) have observed in a stomatopod, *Gonodactylus breedini* that mating is not preceded by moulting.

In spite of the elaborate mating processes reported in some decapod crustaceans, not much is known on the pheromonal attraction among the males. Kittredge *et al* (1971) have pointed out that "the closest parallel to insect pheromone communication observed in marine organisms are the sex pheromones of marine Crustacea." The available information on crustacean sex pheromone indicates that their behavioural assays accepted as admissible evidence for sex pheromone are as follows : (i) chemokinetic reactions, (ii) chemotaxic reactions, (iii) releaser reactions (Dunham 1978). The presence of non-diffusible stimulating substances have been found by Carlisle (1959) and Forster (1951) in *Pandalus borealis* and in *Leander serratus* respectively. They have found that the stimulant is not restricted to any particular part of the body ; instead even antennal contact seems to be sufficient for exciting the male. Ryan (1966) reported on the water soluble sex pheromone released through the urine of *Portunus sanguinolentus* during pre-moult stage. Atema and Engstrom (1971) and McLeese (1971) have also demonstrated the existence of water-soluble sex pheromone released by moulted mature female lobsters. Kamaiguchi (1972) has shown in *Palemon paucidens* that such sex attractant is released from the sternal glands during prepatatorial moult. Sex pheromonal activity of the moulting hormone (crustecdysone) itself has been indicated by Kittredge *et al* (1971). However Atema and Gagosian (1973) found no evidence for the pheromonal activity for ecdysone or its analogue in the mature males. Perhaps the occurrence of sex pheromone in more crustacean species should be demonstrated in order to draw conclusions on its physiological specificity on the males. It is of interest to note in this connection that in a male crab *Emerita asiatica* mating occurs indiscriminately without the involvement of any sex pheromone (Subramoniam 1979). The aim of the present paper is to find out whether the extracts of various female reproductive organs as well as the "female water" possess pheromonal activity on the mature males kept in isolation in the laboratory.

2. Material and methods

Squilla holoschista (Woodmason) used in the present study were collected from the Madras coast and maintained in the laboratory in glass aquaria containing sea water. Water was changed and sufficiently aerated every day. The animals were fed regularly with fresh muscles of fish and prawn.

Behavioural sequences were observed in the glass tanks. Before the experiments commenced the matured males and females were fed *ad libitum* and transferred into a tank of dimensions 60 × 25 × 31 cm, with sufficient sea water.

Before the experiment began the males were fully fed. Then the aqueous extracts of ovary (0.5 g of ovary in 1 ml of filtered sea water), cement glands (0.25 g of cement glands in 1 ml of sea water) and the oviduct (from three animals with 0.5 ml of sea water) were tried on the males when it comes to a motionless state. This was repeated many times in order to find out the changes if any in the behavioural patterns towards the premating gestures or agitated or searching beha-

Table 1. Behavioural sequences

Normal behaviour—Male (In isolation within a period of 30 min)	I. Mating—Male and female (Deecaraman and Subramoniam 1981a) (Generally at evening hrs—diffused light)	Aggressive males (Frequently exhibits)
1. Antennule flicking	1. Antennule flicking—male and female	1. Antennule flicking
2. Spreading of the raptorial meri at narrow angle	2. Contact—male initiates	2. Meri spread out widely
3. Telson thrust	3. Male spreads the raptorial meri female remains motionless	3. Strikes the opponents
4. Forward and backward movements	4. Male holds the female by cephalothoracic appendages, grasps and tilts the female	4. Chase
5. Motionless	5. Male erects the intromittent organs and moves towards the female	
6. Cleaning the cephalo-thorax with telson spines	6. Male exhibits thrusting movements—Female orientates towards the male	Female
7. Coiling by bringing the telson close to the cephalic region	7. Release of male by the female—strikes (indicates to some extent aggressive behaviour)	1. During non-receptive condition 2. Strikes the male at the end of copulation
	II. Repeated mating	
	1. Both male and female involve	
	2. Some behavioural movements repeated as in column 1.	

vioural patterns as reported by Ryan (1966), Atema and Engstrom (1971) and Kamiguchi (1972).

Similarly the effect of "female water" in changing the behavioural pattern of male was also tested. The female water was obtained by keeping a mature female in a glass tank for six hrs. This female water was tested on males kept in isolation in a glass tank. The behaviour of the males after the addition of female water is compared with the normal mating patterns (Deecaraman and Subramoniam 1981b).

3. Results

In *S. holoschista* normal mating behaviour (Deecaraman and Subramoniam 1981b) could be easily differentiated from the aggressive encounters. The male usually exhibits aggressive behaviour. However, the female also exhibits the

same when it is not in the receptive state. This aggressive behaviour by females is also exhibited at the end of copulatory sequences.

The males when introduced into the trough start flicking the antennules in all directions. This movement may last for few sec. Subsequently, the animals spread the raptorial meri on both sides and withdrew them immediately. Then the males move backward using the telson spines and the walking legs. Sometimes, the animals remain motionless up to 10 min, but keep the antennules and the pleopods in motion. Following this the males exhibit forward and backward movements using the thoracic and abdominal appendages, with telson spines planted on the substratum. Frequently the animals clean the maxillipedes with telson spines and also demonstrate "coiling" by bringing the telson and the head close together. These movements may last from a few sec. to some min.

3.1. *Experiments with ovary, oviduct and cement glands extracts*

A mature male was introduced into the tank and its normal behavioural pattern was observed. When the animal comes to a motionless state at one end, the aqueous extract of ovary was introduced at the other end opposite to the animal drop by drop. In the beginning the animal shows a positive response by moving towards the point of application of the ovarian extract; however, it immediately retreats to its original place without showing any behavioural pattern positive to premating gestures. Repeated application of the ovarian extract failed to show any effect on eliciting the premating gestures. Similarly, the application of oviducal and cement glands extracts did not have any effect on the males (table 2).

3.2. *The female water*

To test the effects of female water on the male behavioural pattern the mature males were introduced in the tank. Even here the males failed to elicit any positive behaviour for attraction.

All these preliminary experiments suggest that there may not be any specific stimulation of the male by the female by way of any pheromonal substances (table 2). It is therefore suggested that mating in the stomatopod under laboratory conditions occurs indiscriminately.

4. Discussion

Many available evidences in invertebrates clearly suggest the involvement of pheromone and one such phenomenon is the settlement of marine larvae of gregarious organisms (Dahl 1975; Crisp 1974). Another phenomenon of sex pheromone is that of "epidemic spawning" (Galtsoff 1938, 1940; MacGinite and MacGinite 1949).

Various stomatopod species are known to exhibit agonistic and aggressive behaviour in natural copulation (Dingle and Caldwell 1969, 1976; Caldwell and Dingle 1976). Malacostracans, especially the brachyuran crabs, have been shown to exhibit prolonged premating gestures before the external pairing (Hazelet 1975). Many others have attempted to explain this phenomenon by way of pheromonal

Table 2. Behavioural sequences—Male (within a period of 30 min)

I. Ovary

1. Normal behaviour
2. Motionless
3. Application of ovary extract
4. Antennule flicking
5. Advances towards the point of application
6. Retreats to the normal position immediately
7. No premating gestures or searching behaviour or agitated movements
8. Restore to the normal behaviour

II. Oviduct

1. Normal behaviour
2. Motionless
3. Application of oviduct extract
4. Antennule flicking
5. Avoids the point of application
6. No premating gestures or search behaviour
7. Restore to the normal behaviour

III. Cement glands

1. Normal behaviour
2. Motionless
3. Application of cement glands extract
4. Antennule flicking
5. No premating gestures or searching behaviour or agitated movements
6. Restore normal behaviour

"Female water "

1. Antennule flicking
 2. Motionless
 3. No premating gestures or searching behaviour or agitated movements
 4. Normal behaviour.
-

attraction (Ryan 1966; McLeese 1971; Kittredge *et al* 1971). Virtually nothing is known about the origin of pheromone in the aquatic invertebrates (Dunham 1978). Recently, Kittredge *et al* 1972 and Kittredge and Takahashi (1972) have reported that the crustecdylone or the related compound acts as sex pheromone in some decapod crabs, however Atema and Gagosian (1973) have reported negative results to the sex pheromone response to any one of these compounds in *Homarus americanus*. In *Portunus sanguinolentus* Christofferson (1970) has reported that the sex pheromone is of 1000 or less of molecular weight.

A recent study on the mating behaviour of sand crab *E. asiatica* has shown that the tiny males may not be attracted to the female by any sex pheromone as the attachment of the males to the females occur long before the actual deposition of spermatophore and the attachment could also occur at any time of moult cycle (Subramoniam 1977). It was also reported that the mating at least in this crab is indiscriminate and that there may not be any pheromonal attraction involved in it (Subramoniam 1979). It was also suggested that a pheromone may not work in an environment of rapid water movements such as intertidal region inhabited by *E. asiatica*.

The present results have not provided any evidence in support of a sex pheromonal attraction in *S. holoschista*.

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References

- Atema J and Engstrom D G 1971 Sex pheromone in the lobster *Homarus americanus*; *Nature (London)* **232** 261-263
- Atema J and Gagosian R B 1973 Behavioural responses of male lobsters to ecdysones; *Mar. Behav. Physiol.* **2** 15-20
- Berry P F 1970 Mating behaviour oviposition and fertilisation in the spiny lobster *Panulirus homarus* (Linnaeus); *Invest. Rep. Ocean Res. Inst. S. Afr.* **24** 1-16
- Caldwell R L and Dingle H 1976 Variation of agonistic behaviour between populations of the stomatopod *Haptosquilla glyptocercus*; *Evolution* **31** 220-223
- Carlisle D B 1959 On the sexual biology of *Pandalus borealis*. The termination of the male phase; *J. Mar. Biol. Ass. U.K.* **38** 381-395
- Christofferson J P 1970 *An electrophysiological and chemical investigation of the female sex pheromone of the crab Portunus sanguinolentus* (Herbst.); Ph.D. Thesis, University of Hawaii, Hawaii
- Crisp D J 1974 Studies of barnacle hatching substance; *Comp. Biochem. Physiol.* **30** 1037-1048
- Dahl E 1975 Pheromones in aquatic invertebrates, Bio signals Kungl. Fysiografiska Sallaskapet of Lund, Sweden
- Deecaraman M and Subramoniam T 1981a Repeated mating and its effect on the female reproductive physiology with special reference to the fate of male accessory sex gland secretion; *Proc. First All India Symp. Inv. Repro. Madras*
- Deecaraman M and Subramoniam T 1981b On the question of sex pheromonal attraction in the crustacean stomatopod *Squilla holoschista*; *Abstr. 10th Ann. Conf. Etho. Soc. Bangalore*
- Dingle H and Caldwell R L 1969 The aggressive and territorial behaviour of the mantis shrimp, *Gonodactylus breedini* Manning (Crustacea: Stomatopoda); *Behaviour* **33** 115-136
- Dingle H and Caldwell R L 1972 Reproductive and maternal behaviour of the mantis shrimp *Gonodactylus breedini* Manning (Crustacea: Stomatopoda); *Biol. Bull.* **142** 417-426
- Dingle H and Caldwell R L 1975 Distribution abundance and intraspecific agonistic behaviour of two mudflat stomatopods; *Oecologia* **20** 167-178
- Dunham P J 1978 Sex pheromones in Crustacea; *Biol. Rev.* **53** 555-583
- Forster G R 1951 The biology of the common prawn *Leander serratus* Pennat; *J. Mar. Biol. Ass. U.K.* **30** 333-360

- Galtsoff P S 1938 Physiology of reproduction of *Ostrea virginica* II. Stimulation of spawning in the female oyster; *Biol. Bull.* **75** 286-307
- Galtsoff P S 1940 Physiology of reproduction of *Ostrea virginica*. III. Stimulation of spawning in the male oyster; *Biol. Bull.* **78** 117-135
- Hartnoll R G 1969 Mating in Branchyura; *Crustaceana* **16** 161-181
- Hazlett B A 1970 Tactile stimuli in the social behaviour of *Pagurus bernhardus* (Decapoda, Paguridae); *Behaviour* **36** 20-48
- Hazlett B A 1972 Shell fighting and sexual behaviour in the hermit crab genera *Pagurites* and *Calcinus* with comments on *Pagures*; *Bull. Mar. Sci.* **22** 806-823
- Hazlett B A 1975 Ethological analysis of reproductive behaviour in marine Crustacea; *Publ. Staz. Zool. Napoli.* **39** 671-695
- Hazlett B A and Winn H E 1962 Sound production and associated behaviour of Bermuda crustaceans. (*Panulirus*, *Gonodactylus*, *Alpheus* and *Synalpheus*); *Crustaceana* **4** 25-38
- Kamiguchi Y 1972 Mating behaviour in the freshwater prawn, *Palaemon paucidens*. A study of the sex pheromone and its effect on male; *J. Fac. Sci. Hokkaido Univ.* **18** 347-355
- Karlson P and Lüscher M 1959 "Pheromones": a new term for a class of biologically active substances; *Nature (London)* **183** 55-56
- Kittredge J R, Terry M and Takahashi F T 1971 Sex pheromone activity of the moulting hormone, crustecdysone, on male crabs (*Pachytrapsus crassipes*, *Cancer antennarius*, *C. anthonyi*); *Fish. Bull.* **69** 337-343
- Kittredge J S and Takahashi F T 1972 The evolution of sex pheromone communication in the Arthropoda; *J. Theo. Biol.* **35** 467-471
- MacGinitie G E and MacGinitie N 1949 Natural history of marine animals. (New York, London and Toronto: McGraw-Hill Book Co.) 1-473
- McLeese D W 1971 Detection of dissolved substances by the American lobster (*Homarus americanus*) and olfactory attraction between lobsters; *J. Fish. Res. Canada* **27** 1372-1378
- Nolan B A and Salmon L 1970 The behaviour and ecology of Snapping shrimp (Crustacea: *Alpheus heterochelis* and *Alpheus nomanni*); *Form Funct.* **2** 289-335
- Ryan E P 1966 Pheromone: Evidence in a Decapod crustacean; *Science* **151** 340-341
- Salmon M 1965 Waving display and sound production in the courtship behaviour of *Uca pugilator* with comparison to *U. minax* and *U. pugnax*; *Zoologica* **50** 123-150
- Salmon M 1971 Signal characteristics and acoustic detection by the fiddler crabs, *Uca rapax* and *Uca pugilator*; *Physiol. Zool.* **44** 210-224
- Subramoniam T 1977 Some aspects of sexual biology of a crab *Emerita asiatica* Milne Edwards; *Mar. Biol.* **43** 369-378
- Subramoniam T 1979 Heterosexual raping in the mole crab *Emerita asiatica*; *Int. J. Invertebr. Reprod.* **1** 197-199
- Teytaud A R 1971 The laboratory studies of sex recognition in the blue crab *Callinectes sapidus* Rathbun; *Sea Grant Tech. Bull.* **15** 1-63



A new species of *Argulus* Muller (Crustacea: Branchiura), with a note on the distribution of different species of *Argulus* in India

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Abstract. This paper describes a new species of *Argulus*, *Argulus mangalorensis* collected from the estuarine stretch of Nethravathy river of Mangalore, S. India. The distribution of different species of *Argulus* reported from India is also indicated.

Keywords. *Argulus mangalorensis*, n.sp. description, distribution.

1. Introduction

Two specimens of *Argulus* obtained from the plankton samples from Nethravathy estuary of Mangalore, are found to belong to a new species which is described here. Generally argulids are known to parasitize marine and freshwater fish. The present report records the occurrence of *Argulus* in the estuarine habitats as well. Both the specimens were gravid females and appear to have left their hosts for egg laying. The species of *Argulus* known so far from India are *A. indicus* Weber, *A. giganteus* Ramakrishna, *A. bengalensis* Ramakrishna, *A. siamensis* Wilson, *A. siamensis peninsularis* Ramakrishna, *A. puthenveli* Ramakrishna, *A. siamensis* sub sp. Sundari Bai, *A. fluviatilis* Thomas and Devaraj, *A. cauveriensis* Thomas and Devaraj, *A. japonicus* Thiele and *A. quadristriatus* Devaraj and Ameer Hamsa.

2. Descriptions

Argulus mangalorensis sp. nov. (figures 1-11)

Material: Two gravid females were obtained from the plankton samples from Nethravathy estuary on 3 April 1979. The holotype, a female measuring 8 mm long, will be deposited in the Indian Museum, Calcutta.

Adult female: Body (figures 1, 2) 8 mm long, carapace longer than wide 7.5 × 5.6 mm, anterolateral sinuses distinct, cephalic area 2 mm wide, convex above and spined ventrally, lateral lobes of carapace 5 mm long, rounded behind, spined on anteroventral surface, dorso-median sinus moderately deep, reaching to the level of anterior end of fourth thoracic segment.

The dorsomedial pair of longitudinal ribs of carapace convergent in the middle, curve outward beyond paired eyes anteriorly and below sucker posteriorly, posterior

pieces parallel, end near transverse groove of cephalic region. Each dorsomedial rib bears a pair of sutures at about its middle region, a pair of longitudinal sutures arise from below compound eyes, run sideways and proceed backwards and join the transverse groove of cephalic region. Secondary sutures arise from the triangular sutures, extend backward, parallel to the lateral lobes of carapace and reach almost at the level of base of fourth thoracic segment, and are connected with each other by a transverse groove. None of the sutures are marked by any pigments or coloured stripes.

Abdomen is 2.6×3.0 mm, truncate anteriorly, posterior lobes subacute, sinus deep, narrow anteriorly, and broad posteriorly. Caudal rami small, each with three terminal subequal setae.

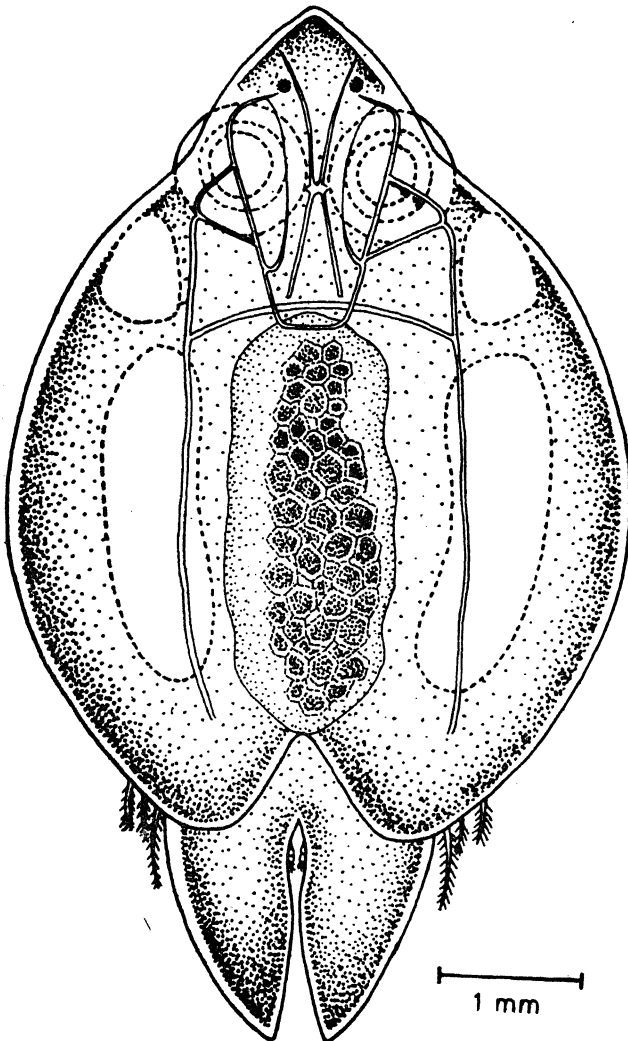


Figure 1

The anterior respiratory areas oval, equal in size, found near lateral margins of carapace between the level of base of suckers and maxillipeds. Posterior respiratory areas elongated, kidney-shaped, found between the level of origin of first thoracic segment and base of fourth thoracic segment.

Basal segment of first antenna with a strong medial outcurved spine, next segment with a stout spine and antennular spine strongly curved. Slender terminal segment of antenna with three minute spines and setae distally (figures 3, 4). Second antenna (figure 5) four segmented, basal segment broad with a stout spine at base, six setae on dorsal and four on ventral margins. Second segment elongated with four setae of which two are on dorsal margin, remaining two at distal seta. Fourth segment small, club-shaped with three small equal apical seta. Fourth segment small, club-shaped with three small equal apical

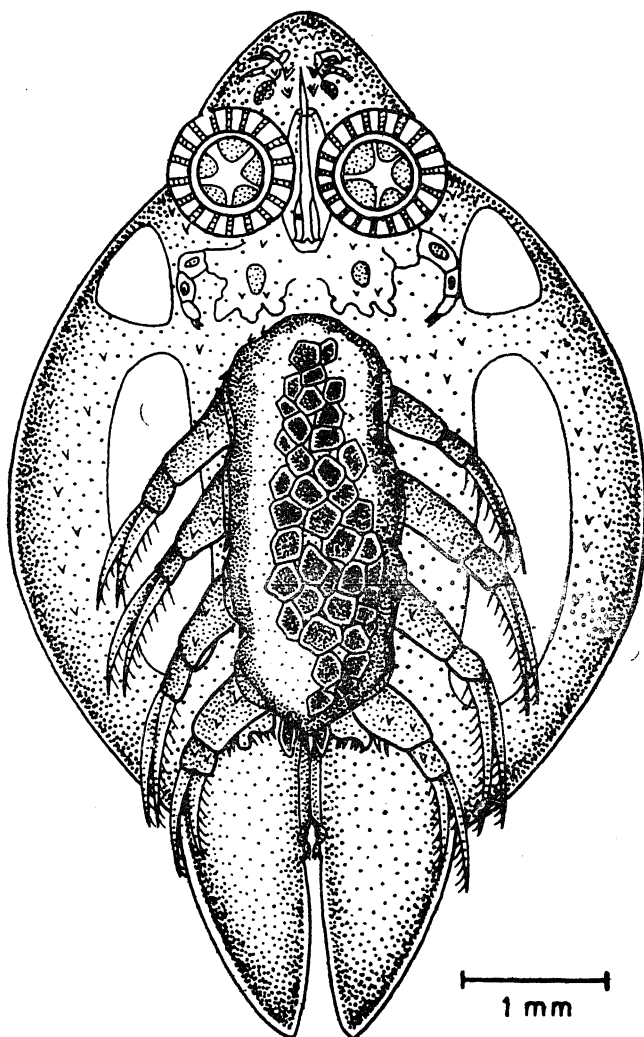
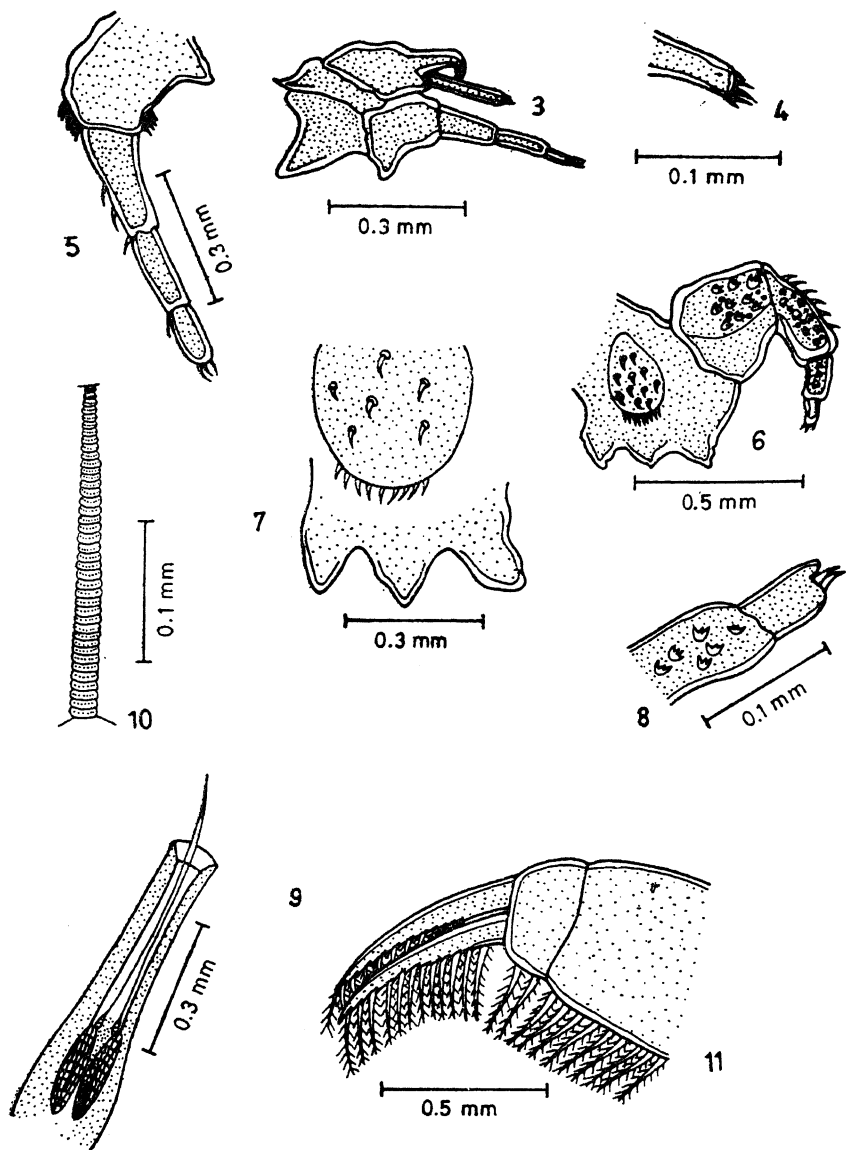


Figure 2



Figures 3-11

Figures 1-11. *Argulus mangalorensis* sp. nov. 1. dorsal view; 2. ventral view; 3. first and second antenna; 4. distal end of first antenna enlarged; 5. second antenna enlarged; 6. maxilliped; 7. basal segment of maxilliped enlarged; 8. distal segment of maxilliped enlarged; 9. stylet; 10. single rib with plates; 11. fourth leg.

spines. Postantennal spine very stout, maxilliped (Fig 6) five segmented, basal segment with three nearly equal stout posteromedial spines and a large oval spinous pad which carries nine setae along its setae. Second and third segments are provided with rectangular pads which carry scale-like spines and a row of

Table 1. Distribution of species and sub-species of *Argulus* from India.

Parasite	Host	Locality	Author and Year
<i>A. siamensis</i>	Not known	Harischandrapur, W. Bengal	Ramakrishna 1951
	<i>Ophiocephalus</i>	Champahati,	
	<i>punctatus</i>	W. Bengal	
	<i>Labeo rohita</i>	Siripur, Bihar	
	Not known	Mahananda River, Base of Himalayas	
	Murrel	Saurashtra	Ramakrishna 1951
<i>A. indicus</i>	<i>Ophiocephalus</i>	Champahati,	
	<i>punctatus</i>	W. Bengal	
<i>A. giganteus</i>	Not known	Not known	Ramakrishna 1951
	<i>Tetradon oblongus</i>	Bombay	Rangnekar 1957
<i>A. bengalensis</i>	Not known	Harischandrapur, W. Bengal	Ramakrishna 1951
<i>A. siamensis</i> sub sp.	Not known	Rajahmundry	Ramakrishna 1951
<i>peninsularis</i>	Ambassis ranga	Rajahmundry	Malaviya 1955
<i>A. puthenveliensis</i>	Not known	Not known	Ramakrishna 1962
	<i>Esomus danrica</i>	Kerala	Thomas 1961
	<i>Puntius vittatus</i>		
	<i>Macropodus eupanus</i>		
	<i>Panchax panchax</i> <i>blochii</i>		
<i>A. siamensis</i> sub sp.	<i>Lebistes reticulatus</i>	Hasaragatta, Bangalore	Sundari Bai 1973
<i>A. fluviatilis</i>	Not known	Hoginekal, Tamil Nadu	Thomas and Devaraj 1975
<i>A. cauveriensis</i>	Not known	Hoginekal, Tamil Nadu	Thomas and Devaraj 1975
<i>A. japonicus</i>	<i>Labeo fimbriatus</i>	Sathanur fish farm,	Prabhavathy and
	<i>Catla catla</i>	Tamil Nadu	Sreenivasan 1976
	<i>Cyprinus carpio</i>		
<i>A. quadristriatus</i>	<i>Psammoperca</i> <i>waigiensis</i>	Palk Bay, Mandapam	Devaraj and Ameer Hamsa 1977

similar spines on the margin of the third segment. Fourth segment which is smaller than the third, also carries spines. Fifth segment small with a blunt lobular distal end and two dissimilar claws on inner margin.

Paired lateral eyes conspicuous, located at base of antennal spine, median eye well developed, proboscis midventral, in between suckers. Distal half of proboscis expanded, anterior part narrow, terminating in a stylet (figure 9). Sucker 0.6 mm (inside diameter), composed of 115-118 ribs of 38 to 40 imbricated plates each (figure 10).

Distal ends of rami of third and fourth legs reach a little beyond carapace. Flagella of swimming legs absent, basal lobe of fourth leg boot-shaped, carries setae on ventral margin, basal segment of basipod with nine and distal segment with two plumose setae (figure 11). The thoracic segments and basipods with spines ventrally. Uterine eggs are four to five sided, arranged, in honey-comb pattern.

Colour : Body greenish yellow, papillae algal green, thoracic segments and legs straw yellowish and uterine eggs dull brown.

3. Discussion

In the arrangement of respiratory areas, *A. mangalorensis* agrees with 22 species of *Argulus* in Wilson's (1944) report. *A. kusafugu* and *A. scutiformis* from Japanese fishes (Yamaguti and Yamasu 1959), *A. indicus* and *A. giganteus* from India (Ramakrishna 1951), *A. japonicus* from pond fishes of Tamil Nadu (Prabhavathy and Sreenivasan 1976) and *A. quadristriatus* from a marine fish (Devaraj and Ameer Hamsa 1977). In the arrangement of the respiratory areas as well as the suction cup being composed exclusively of imbricated plates, *A. mangalorensis* is similar to *A. melanosticus*, *A. pugettensis*, *A. niger*, *A. floridensis* and *A. giganteus* and *A. quadristriatus*. However, the present species is distinct from the others by the following characteristics—(1) 115–118 number of ribs in each suction cup ; (2) 38–40 imbricated plates in each rib ; (3) three spines and three setae at the distal end of first antenna ; (4) absence of flagella on any of the swimming, legs.

The distribution of *Argulus* spp. in India is given in table 1.

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References

- Devaraj M and Ameer Hamsa K M S 1977 A new species of *Argulus* (Branchiura) from a marine fish, *Psammoperca waigiensis* (Cuvier) ; *Crustaceana* 32 129–134
- Malaviya R B 1955 Parasitism of *Ambassis ranga* H.B. by *Argulus siamensis* sub sp. *peninsularis* Ramakrishna ; *Curr. Sci.* 24 275
- Prabhavathy G and Sreenivasan A 1976 Occurrence of *Argulus japonicus* in brood fish ponds in Tamil Nadu ; *J. Inland Fish. Soc. India* 8 131–133
- Ramakrishna G 1951 Notes on the Indian species of the genus *Argulus* Muller (Crustacea : Copepoda) parasitic on fishes ; *Rec. Indian Mus.* 49 207–216
- Ramakrishna G 1962 On a new species of *Argulus* Muller (Crustacea : Copepoda) from Kerala ; *Proc. All India Congr. Zool.* 1 178–179
- Ranganekar M P 1957 Copepod parasite of the families Argulidae, Caligidae, Dichelesthidae and Lernaeopodidae ; *J. Univ. Bombay* 26 8–20
- Sundari Bai A 1973 The occurrence of Branchiura parasite *Argulus* sp. (Argulidea : Argulidae) on the carnivorous fish *Lebistes reticulatus* (Peters) In Mysore State ; *Curr. Res.* 2 79–75
- Thomas M M 1961 Observations on the habits and post-embryonic development of a parasitic branchiuran *Argulus puthenveliensis* Ramakrishna ; *J. Mar. Biol. Ass. India* 3 75–86
- Thomas M M and Devaraj M 1975 Two new species of *Argulus* Muller (Crustacea : Branchiura) from River Cauvery with a key to Indian species ; *Indian J. Fish.* 22 215–220
- Wilson C B 1944 Parasitic Copepoda in the United States National Museum ; *Proc. U.S. Nat. Mus.* 94 529–582
- Yamaguti S and Yamasu T 1959 On two species of *Argulus* (Branchiura : Crustacea) from Japanese fishes ; *Biol. J. Okayama Univ.* 5 167–175

The effect of cephalic transection on the micromorphological changes in the ventral nerve cord-neurosecretory system of earthworm, *Metaphire peguana* (Rosa, 1890) during anterior regeneration

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Abstract. Transection of anterior 5 segments in *Metaphire peguana* engenders characteristic changes in the functional activity of the ventral nerve cord-neurosecretory system in the event of cephalic regeneration. Of the two types of neurosecretory cells, the moderately stained cells remain more susceptible when the cell structure, location of nucleus, amount of secretory inclusions and their transportation to the zone of accumulation are considered. Overall engorgement of neurosecretory substances refrained from axonal transport, moderate axonal flow coupled with slight depletion and finally acute depletion at 24, 48 and 72 hr after amputation respectively are some of the notable features registered in course of this investigation. Disarray in the sequential changes involved in the secretory dynamics of neurosecretory cells, as well as extent of NSM accumulation both within and outer periphery of the ganglia provide evidence for the utilisation of material through repaired vascular systems during regenerative proliferations of anterior segments.

Keywords. *Metaphire peguana*; neurosecretory cells; NSM; regeneration; secretory dynamics.

1. Introduction

The importance of the central nervous system in the phenomenon of oligochaete regeneration has been elucidated by several classical investigators (Morgan 1902 ; Avel 1929 ; Sayles 1940). Harms (1948) experimentally established the indispensability of the brain for regenerative growth of the anterior segments in *Lumbricus terrestris*. Later Herlant-Meewis (1964) refuted the solitary role of the cerebral ganglia and advocated the involvement of the ganglionic complements of the ventral nerve cord in both anterior and posterior regeneration in *Eisenia foetida*. These observations have been substantiated by Farber (1965) who reiterated that neurosecretion of the ventral nerve cord has a profound role in the segmental regeneration of *L. terrestris*. In her detailed analysis, Herlant-Meewis (1972) opined that C₃ cells of each segmental ganglion exhibit spectacular cytological response to the loss of anterior segments. Synchronous release and synthesis of neurosecretory material (NSM) in the ganglia immediately proximal

to the level of amputation of either anterior or posterior segments in *E. foetida* have been recorded by Marcel (1973) who also concluded that neurosecretory system promotes some aspects of regeneration.

The present investigation deals with the extent of histomorphic changes in the neurosecretory system of the ventral ganglionic complements following anterior amputation. An attempt has also been made to assess sequential reactive response in the ganglia concerned.

2. Materials and methods

Full grown clitellate earthworms, *Metaphire peguana* (average length 120 mm) were collected from the neighbourhood of Calcutta and acclimated for one week in the laboratory at room temperature 29° C and RH 78%. Amputation of the first five anterior segments by a sterilized paragon knife was made in the group comprising fifteen worms which were kept in a petridish containing 1.5 inch thick bed of moisturized soil. Ganglionic complements from anterior, middle and posterior regions (each region containing 40 segments) of the remaining nerve cord were fixed in Bouin's fluid after 24, 48 and 72 hr of amputation. Identical sets of ganglionic complements were dissected out from unoperated earthworms which, however, served as controls. Sections (7 μ m thick) were stained with both Gomori's chromealum-haematoxylin phloxin (Bargmann 1949) and simplified aldehyde fuchsin (Cameron and Steele 1959) staining techniques following acid permanganate oxidation.

3. Observations

3.1. Control

A majority of the neurosecretory cells of the ventral nerve cord are in various phases of secretion (figure 1a) which can be determined on the basis of the staining intensities in descending order to locate the concentration of secretory material. Relatively small deep stained cells do not exhibit detectable cytoplasmic inclusions and usually possess more or less homogeneously stained cytoplasm (figure 1b). Large moderately stained cells, however, possess variable amount of secretory inclusions apart from clarity in their cytoarchitecture (Nanda and Chaudhuri 1981). Some of the moderately stained cells exhibit axonal transport of secretory material and their subsequent discharge. Evidence for rich NSM accumulation both at the margin of the neuropile, as well as, the outer periphery of the ganglia as such are not seldom.

3.2. Experimental

Appearance of regeneration blastema is first noticed within 24–48 hr after cephalic transection (Nanda and Chaudhuri 1982). Completion in the formation of a full fledged anterior segment, however, is accomplished around 72 hr after amputation. Such operation renders multiple cytomorphic alterations that are

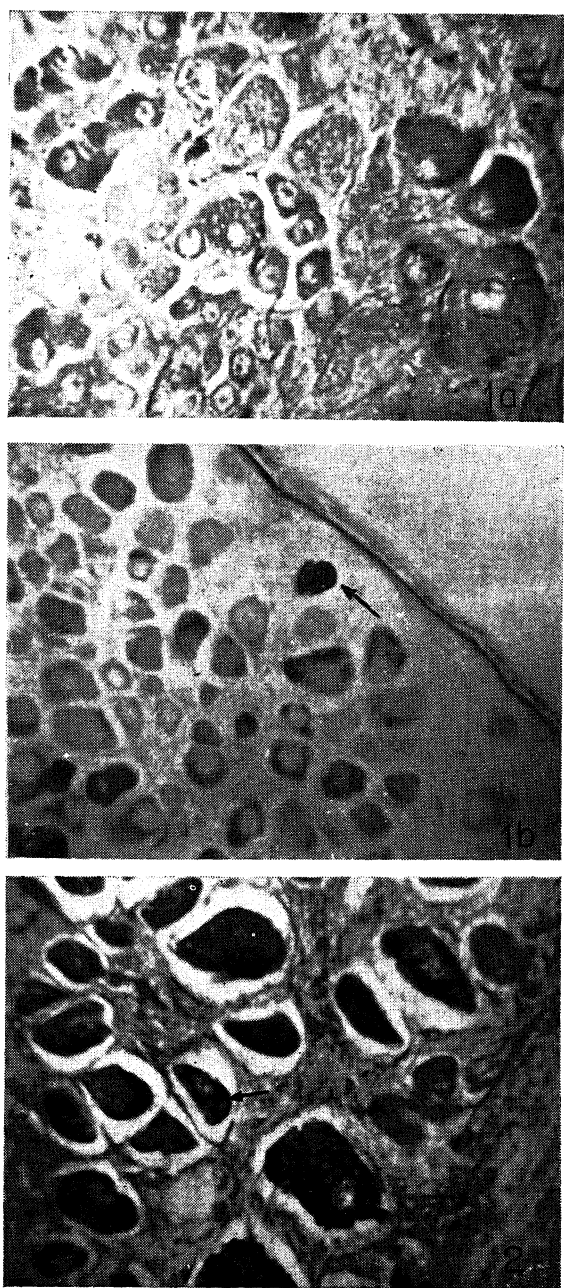


Figure 1. (a) Control section of the ventral nerve cord of *Metaphire peguana* showing CHP-positive neurosecretory cells with various phases of secretory activity ($\times 1500$). (b) Control section showing AF-positive cytoplasm of deep and moderately stained cells. Note homogeneously stained cytoplasm of deep stained cell ($\times 1500$).

Figure 2. Experimental: Section showing typical "shrunk condition" of CHP-positive moderately stained cells in the ventral nerve cord following anterior transection ($\times 1500$).

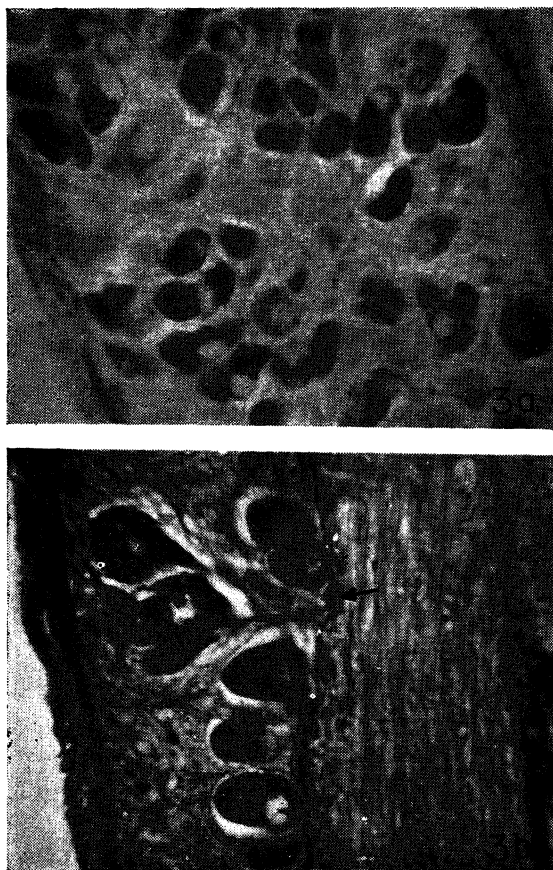


Figure 3. Experimental: (24 hr after anterior transection.) (a) Section showing trend in the massive accumulation of AF-positive material in the neurosecretory perikarya ($\times 1500$). (b) Section showing some moderately stained cells with secretion in the form of aggregates and axon oriented nuclei. Note discrete accumulation of CHF-positive material at the margin of neuropile ($\times 1500$).

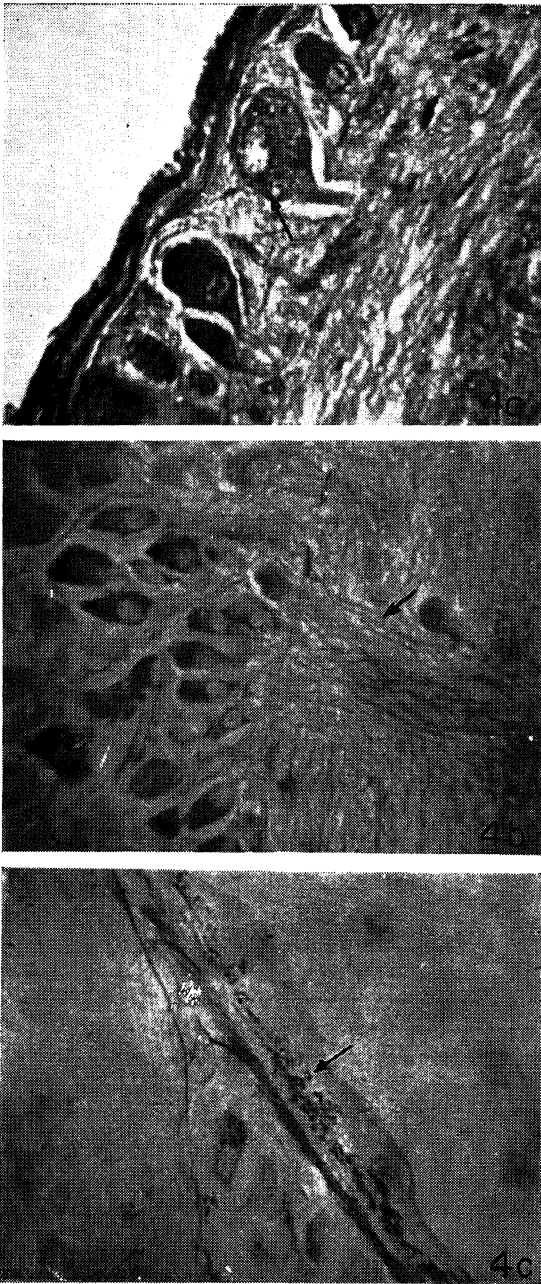


Figure 4. Experimental: (48 hr after anterior transection). (a) Section showing CHP-positive moderately stained cells with cytoplasmic vacuoles in the perikarya ($\times 1500$). (b) Section showing AF-positive cells discharging their secretory material through 'axon bundle'. Note axon oriented nuclei ($\times 1500$). (c) Section showing accumulation of AF-positive secretory colloids at the peripheral margin of the ganglion ($\times 1500$).

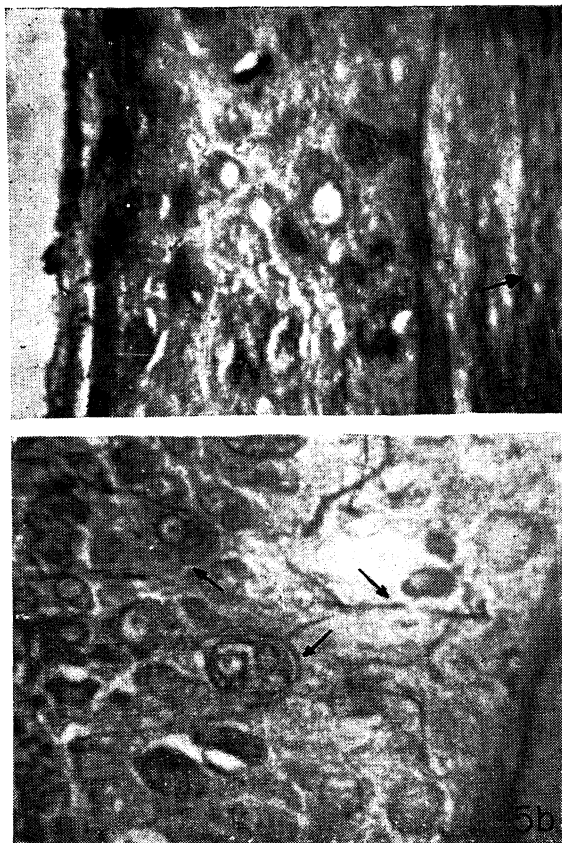


Figure 5. Experimental: (72 hr after anterior transection.) (a) Section showing sudden drop in staining intensity of AF-positive secretory neurones. Note contrasting staining feature of the neuropile due to accumulation of secretory material ($\times 1500$). (b) Section showing CHP-positive neurosecretory cells with cytoplasmic vacuoles. Note ramification of intraganglionic blood vessels endowed with secretory inclusions. ($\times 1500$),

more apparent in case of moderately stained cells. These cells in contrast with the deep stained cells also reveal conspicuous "shrunk conditions" of the cell body (?) which is pronounced up to 48 hr of experimentation (figure 2).

3.3. 24 hr after amputation

Most of the neurosecretory cells irrespective of their types especially of the anterior region of the ventral nervecord show intense accumulation of secretory inclusions in their perikarya (figure 3a). These inclusions may exist in the form of close aggregates so as to render the cytoplasm coarse in appearance. This condition is rather predominant in moderately stained cells which have aggregates mostly concentrated at the anterior half of the perikarya. Nuclei, however, are often observed more towards the axon hillock region. The margin of the neuropile remains sprinkled with secretory inclusions (figure 3b).

3.4. 48 hr after amputation

Despite deep stainability in the majority of the neurosecretory cells in the ventral nerve cord a few cells are in a state of depletion and vacuoles in the perikarya are not scarce (figures 2 and 4a). Majority of the cells bear axon oriented nuclei with brilliant phloxinophilic nucleoli and exhibit axonal transport (figure 4b). In contrast, the neuropile falls short of NSM while enhanced accumulation of AF-positive material is obvious at the outer periphery of the ganglia (figure 4c).

3.5. 72 hr after amputation

The general trend for the deep stainability of cells demonstrating discrete secretory inclusions as found above suddenly declines (figure 5a). A few deeply stained cells still exist but they do not demonstrate coarse cytoplasm. On the other hand, they remain homogeneously stained and are comparable to those of the control. Moderately stained cells are very clear and they exhibit intense vacuolation in their perikarya which often are devoid of any cytoplasmic inclusions. Occasional axonal transport throughout ganglionic complements of the ventral nerve cord may be noticed. Incidentally, the margin of the neuropile, as well as the intra-ganglionic blood vessels show secretory inclusions but the peripheral region of the ganglia demonstrate very little NSM (figure 5b).

4. Discussion

Cephalic transection on the rest of the ganglia of the ventral nerve cord in *M. peguana* has elicited altered secretory activity in the neurosecretory cells especially when the position of the nuclei, the concentration of cytoplasmic inclusions and the rate of axonal migration of NSM are considered. Such oscillation in the functional activity may have correlation with their spectacular 'hyper-activity' (Herlant-Meewis 1964). Changes in the neurosecretory cells are most conspicuous 24 hr after amputation, close to the level of transection than at other regions. But thereafter almost uniform changes are noticed throughout the nervecord at 48 and 72 hr after amputation. The reason is not clear and

may have bearing in relation to the intensity of stress in course of segmental proliferation. Causes for the shrunken conditions of some neurosecretory cells in general and moderately stained cells in particular are not understood but involvement of "generalised stress action" as reiterated by Farber (1965) could be the reasons. Further, the disarray in sequential changes in the neurosecretory perikarya following transection of ventral nervecord of *M. peguana* incur disruption in neurohormonal balance so as to trigger temporary accumulation of secretory substances in all the neurosecretory cells (Herlant-Meewis 1964). In fact, temporary cessation of neurosecretory transport has some bearing in the event of restitution and subsequent blastema formation. Participation of the NSC_s to discharge their elaboration either partially or indiscriminately into the just repaired circulatory system at late post-amputation periods, i.e. at 48 and 72 hr provides clue for their indispensability in the management of restoration of lost part or "replacement of element" during regeneration (Herlant-Meewis 1962; Dey and Nanda 1979). Indeed, increment in the number of moderately stained cells with spectacular intracellular changes at 72 hr post anterior transection period in contrast to accelerated rate of axonal transport and initiation in the transformation of the moderately stained cells at 48 hr post-amputation period seem to indicate functional change over in the secretory dynamics of the deep and moderately stained cells. Relatively rich accumulation of NSM around the periphery of the neuropile, at the initial stage of experimentation and subsequent exhaustion of the same at 48 hr followed by massive accumulation at 72 hr of post-amputation tend to indicate fluctuation in the secretory rhythm pertaining to increased axonal flow. In consequence, rapid disposal of NSM in the intraganglionic capillaries to mitigate restorative response and thereafter resumption to near-normal condition ensure. Adverse physiological stress condition in the form of injury possibly release cellular products that act as an adjunct to stimulate the neurosecretory neurones of the ventral nerve cord for the production of "regeneration promoting hormones" leading to segment proliferation (Hoar 1975). Besides these, non-existence of discrete non-neural endocrine gland, as well as distinct neurohaemal organ in oligochaetes in general and *M. peguana* in particular, it is reasonable to assume that nervous system as a whole plays a "versatile" role to meet altered physiological eventualities.

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References

- Avel M 1929 Recherches expérimentales sur les caractères sexuels somatiques des Lumbriciens; *Bull. Biol. France et Belg.* 63 149-318
- Bargmann W 1949 Über die neurosekretorische Verknüpfung von Hypothalamus und Neurohypophyse; *Z. Zellforsch.* 34 610-634

- Cameron M L and Steele J E 1959 Simplified aldehyde fuchsin staining of neurosecretory cells ; *Stain Tech.* **34** 265-266
- Dey M and Nanda D K 1979 Effect of posterior transection on the brain neurosecretory perikarya of *Pheretima posthuma* ; *Zool. Beitr.* **25** 199-204
- Farber P A 1965 The histological relationship between neurosecretory activity and anterior regeneration in *Lumbricus terrestris* ; *Anat. Rec.* **151** 348 (Abs.)
- Harms J W 1948 Über ein inkretorisches Cerebralorgan bei Lumbriciden sowie Beschreibung eines verwandten Organs bei drei neuen Lycastis-Arten ; *Arch. Entw.-Mech.* **143** 332-346
- Herlant-Meewis H 1962 Neurosecretory phenomena during regeneration of nervous centres in *Eisenia foetida* ; *Mem. Soc. Endocrinol.* **12** 267-274
- Herlant-Meewis H 1964 Regeneration in Annelids ; *Adv. Morphog.* **4** 155-216
- Herlant-Meewis H 1972 Le rôle du système nerveux dans la cicatrisation chez *Eisenia foetida* (abst.) ; *Gen. Comp. Endocrinol.* **18** 596
- Hoar W S 1975 *General and Comparative Physiology* (New Delhi : Prentice Hall of India Private Limited) pp 752-753
- Marcel R 1973 Cycle sécrétoires de cellules de la chaîne nerveuse au cours de la régénération chez *Eisenia foetida* Sav. f. typica (Annélide, Oligochaète) ; *Gen. Comp. Endocrinol.* **21** 30-44
- Morgan T H 1902 Experimental studies of the internal factors of regeneration in the earthworm ; *Arch. Entw.-Mech. Org.* **14** 562-591
- Nanda D K and Chaudhuri P S 1981 Studies on the cytomorphology of the ventral nervecord of the earthworm, *Pheretima posthuma* with special reference to neurosecretion ; *J. Zool. Soc. India* (in press)
- Nanda D K and Chaudhuri P S 1982 Regeneration of the neurosecretory system of the nerve ring in earthworm, *Metaphire peguana* ; *Acta Biol. Cracov.* (in press)
- Sayles L P 1940 Buds induced by implants of the anterior nerve cord and neighbouring tissues inserted at various levels in *Clymenella torquata* ; *Biol. Bull.* **78** 298-311

Studies on preference of *Callosobruchus maculatus* Fabricius to some high yielding varieties of arhar (*Cajanus cajan* L.)

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Abstract. The oviposition response and development of *Callosobruchus maculatus* Fabricius were studied on 14 high yielding varieties of arhar. There was significant difference among the varieties in the amount of food consumed per grub. The average development period was not dependent on the amount of food consumed. The development of grub was also not better on the grain preferred by the beetle for oviposition. There was significant difference among the varieties in the loss of 100 seed weight. Average weight of female was more than the male developed on all varieties. On the basis of food consumed per grub and loss of 100 seed weight as a combined criterion, the varieties are grouped into least susceptible, intermediate in susceptibility and the most susceptible varieties.

Keywords. Varietal preference ; *Callosobruchus maculatus*.

1. Introduction

Storage of pulse seeds is a problem owing to the severe damage caused by the pulse beetle, *Callosobruchus maculatus* Fabricius. The damage is sometimes so serious that whole of the seed material is eaten and only thick seed coat with empty cavities are left behind. Gokhale (1973), Wadnerker *et al* (1978) and Dabi *et al* (1979) assessed the relative susceptibility of some varieties of different pulses to *C. maculatus*. Attempts have also been made to investigate the cause of differential response of different pulses on various life processes of this beetle (Girish *et al* 1974). But the available literature reveals that practically no attention has been paid towards the susceptibility of high yielding varieties of arhar under cultivation to *C. maculatus*. The present investigation was therefore undertaken.

2. Materials and methods

Fourteen varieties of arhar (*Cajanus cajan* L.) were obtained from the Chief Scientist, Dry Farming, Central Arid Zone Research Institute, Jodhpur. Healthy and uncontaminated seeds were sterilized and the moisture contents of seeds were maintained between 12.5 to 13.0%. 100 seeds of each variety were weighed and kept in plastic vial (5 × 4 × 3 cm). The experiment was replicated five times.

Four pairs (4 males + 4 females) of newly emerged adults from uniparental culture were introduced into each vial except the fifth replication, which was kept without beetle as control for each variety. After 10 days the beetles were removed and the number of eggs laid on each variety was counted. All the experiments were carried out in an incubator at a constant temperature of $28 \pm 2^\circ \text{C}$ and humidity 50–60% r.h.

Commencing from the 20th day of the experiment, the newly emerged beetles were counted daily till the emergence of last adult. After each observation the emerged beetles were removed to prevent further breeding. The weight of seeds and adults were recorded separately with a single pan electric balance (with 0.1 mg precision). The average development period and percentage emergence of adults was calculated. All the data were statistically analysed. The correlation coefficient (r) was calculated between the various life processes of the beetle and physical characters of seed to establish possible relationship between them.

3. Results and discussion

The results (table 1) reveal that all the varieties of arhar were utilized by the beetle for egg laying. The response of oviposition however varied significantly. Varieties 4-84, 4-64, BS. 1, K-28 and T-7 (with average of 238.25 to 273.25 eggs) showed preference for oviposition as compared to variety T-17 (with average of 170.00 eggs). There was no significant difference in the rest of the varieties where the average number of eggs laid varied from 199.50 to 229.75. The minimum number of eggs laid per seed was 1.70. The correlation coefficient (r) between the average number of eggs laid and the seed characters, viz., seed weight, seed volume and colour of seed was not significant (table 2). Further, the texture of seed cannot be taken as a criterion for the preference for oviposition as the texture was smooth in all the varieties tested.

The average food consumed per grub is a good criterion for the assessment of relative susceptibility of different varieties (Regupathy and Rathinaswamy 1970; Dabi *et al* 1979). There was significant difference among the varieties in the amount of food consumed per grub (table 1). Varieties HP (WP)-15, T-17, K-16, B.S. 1, T.T. 4 and 4-64 were least susceptible to *C. maculatus* (with 30.69 to 34.39 mg of food consumption per grub) than the other varieties. The correlation coefficient (r) between the amount of food consumed per grub and the seed characters, viz, seed weight, seed volume and colour of seed was not significant (table 2). Similar observations were reported in the experiment with *Callosobruchus chinensis* reared on different varieties of pigeonpea (Regupathy and Rathinaswamy 1970) and with *C. maculatus* reared on different varieties of cowpea (Dabi *et al* 1979). Apparently some factor other than seed characters governs the mechanism of resistance in pulse seed to the attack of pulse beetle.

The average development period was found to vary significantly which ranged from 27.82 to 34.71 days (table 1). Coefficient of correlation (r) between the amount of food consumed per grub and the average development period was not significant (table 2). The study thus reveals that the development period of the grub is not dependent on the amount of food consumed. Further, the development of grub was also not better on the grain which were preferred by the beetle for oviposition (table 1). Thus the preference for oviposition is not an indication

Table 1. Differential preference of the pulse beetle, *C. maculatus* to different varieties of arhar.

Variety	Number of eggs laid/100 seeds	Food consumed/grub (mg)	Loss of weight/100 seeds (g)	Average development period (days)	Percentage emergence of adult	Average weight of adult (mg)		Average weight of 100 seeds (g)	Number of seeds/10 ml volume
						Male	Female		
'HP (W.P.)-15'	199.50	30.69	2.765	34.71	40.30 (41.70)*	7.44	8.76	7.889	100
'T-17'	170.00	32.42	2.775	29.69	40.55 (40.71)	7.20	8.36	8.904	86
'K-16'	228.25	32.79	3.059	30.36	40.85 (39.72)	7.60	8.80	9.604	78
'B.S.1'	246.00	32.92	3.046	28.83	37.90 (37.98)	7.32	8.88	7.393	87
'T.T.4'	229.75	34.09	3.266	29.85	40.07 (39.69)	7.32	8.60	8.640	87
'4-64'	240.00	34.39	3.227	30.37	39.80 (39.08)	7.08	8.80	7.253	107
'K-28'	251.25	34.51	3.077	29.75	38.05 (38.08)	7.40	9.16	9.091	79
'T.T.2'	213.25	35.23	3.243	28.29	43.17 (41.07)	7.40	9.00	7.610	96
'K-23'	227.75	35.36	3.231	30.63	40.22 (39.35)	7.28	9.36	9.260	73
'4-84'	238.25	35.83	3.407	27.82	40.42 (39.45)	7.36	9.24	7.572	94
'T.T.6'	211.75	36.20	3.391	30.23	44.72 (41.95)	7.72	9.20	8.952	80
'T-7'	273.25	37.66	3.579	30.29	46.15 (42.35)	7.92	9.76	10.430	76
'P.S.41'	224.75	37.87	3.428	32.28	47.67 (43.67)	7.64	9.56	9.663	71
'Basant'	221.75	41.00	3.638	32.78	52.20 (46.26)	7.84	9.48	10.499	64
S.Em.	±16.97	±0.97	±0.126	±0.31	±1.18	±0.35	±0.33		
C.D. at 5%	48.52	2.79	0.366	0.89	3.37	1.02	0.96		
C.D. at 1%	64.92	3.73	0.490	1.19	4.51	1.75	1.26		

* Figures in parenthesis are angular transformed values.

Table 2. Coefficient of correlation (r) between physical characters of seed and life processes of the beetle.

	Average weight of 100 seeds	Average number of seeds/10 ml volume	Colour of seed	Average development period
Average number of eggs laid	0.137	-0.165	0.210	..
Level of significance	NS	NS	NS	
Average food consumed/grub	0.588	-0.628	-0.125	0.168
Level of significance	NS	NS	NS	NS

NS = Not significant.

of suitability for development. These observations are in accordance with the findings of Girish *et al* (1974) and Singh *et al* (1977). The loss of 100 seed weight varied from 2.765 to 3.638 g. Varieties Basant, PS-41, T-7, T.T.6, T.T.-5, 4-84 showed significantly greater loss in seed weight as compared to HP (WP)-15, T.T.17, K-16, B.S.1 and K-28 at C.D. value of 0.05%. The percentage emergence of adults on varieties Basant and PS-41 was significantly more than of the other varieties. Average weight of female was more than the male emerged on all the varieties tested in the present investigation. Similar tendency was observed earlier by Howe and Currie (1964) and Gokhale (1973).

From the overall results on the basis of average food consumed per grub of emerged beetles and loss of 100 seed weight as a combined criterion, varieties HP (WP) -15, T-17, K-16 and B.S. 1 proved to be the least susceptible whereas T.T.6, T-7, PS-41 and Basant as the most susceptible varieties. The varieties T.T.4, 4-64, K-28, K-23, T.T.2 and 4-84 are intermediate in susceptibility and none of the varieties was found immune to the attack of *C. maculatus*.

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References

- Dabi R K, Gupta H C and Sharma S K 1979 Relative susceptibility of some cowpea varieties to pulse beetle *Callosobruchus maculatus* Fabricius; *Indian J. Agric. Sci.* 49 48-50
- Girish G K, Singh Karan and Krishnamurthy K 1974 Studies on the oviposition and development of *Callosobruchus maculatus* Fab. on various stored pulses; *Bull. Grain Tech.* 12 113-116

- Gokhale V G 1973 Development compatibility of several pulses in the Bruchidae. 1. Growth, and development of *Callosobruchus maculatus* Fabricius; *Bull. Grain Tech.* **11** 28-31
- Howe R W and Currie J E 1964 Some laboratory observations on the rate of development, mortality and oviposition of several species of Bruchidae breeding; *Bull. Entomol. Res.* **55** 437-477
- Regupathy A and Rathinaswamy R 1970 Studies on comparative susceptibility of seeds of certain red gram [*Cajanus cajan* (L.)] Mill sp. varieties to pulse beetle, *Callosobruchus chinensis* (L.) (Bruchidae; Coleoptera); *Mad. Agric. J.* **57** 106-109
- Singh Suchwant, Odak S C and Singh Zile 1977 Studies on the preference of pulse beetle (*Callosobruchus chinensis* Linn.) for different hosts; *Bull. Grain Tech.* **15** 20-26
- Wadnerkar D W, Kaunsale P P and Pawar V M 1978 Studies on preference of pulse beetle *Callosobruchus maculatus* Fab. to some varieties of arhar and gram; *Bull. Grain Tech.* **16** 122-124

Three new species of haematozoans from freshwater teleosts (pisces)

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Abstract. Two new species of haematozoans, *Trypanosoma aori* (sp. nov.) and *Trypanoplasma mysti* (sp. nov.), were found harbouring the blood plasma of freshwater teleosts, *Mystus aor*, while *Trypanoplasma atti* (sp. nov.), was found in the plasma of another cat fish *Wallago attu*. The two hosts are new records for these parasites. All three species of the parasites described here showed characteristic polymorphism.

Keywords. Haematozoa; *Trypanosoma*; *Trypanoplasma*; blood; polymorphism.

1. Introduction

In the recent past quite a few new species of piscine haemoflagellate parasites have been described from various freshwater teleosts of India (Ray Chaudhuri and Misra 1973; Misra *et al* 1973; Tandon and Joshi 1973; Pandey and Pandey 1974; Mandal 1975, 1977, 1978, 1979 and Joshi 1976, 1978), besides the earlier reports (Lingard 1904, Demello and Valles 1936, Qadri 1955, 1962 and Hasan and Qasim 1962). In a recent paper Joshi (1979) reported occurrence of trypanosomes in thirteen species of freshwater teleosts of Lucknow. This paper describes three new species of haematozoan parasites from two freshwater teleosts viz. *Mystus aor* and *Wallago attu*.

2. Material and methods

Live specimens of *M. aor* and *W. attu* were obtained from river Gomati, transported to the laboratory, given rest for 12-14 hr in a large glass aquarium under laboratory conditions and then studied the blood smears, stained with Leishman and Wrights stains following the usual methods described earlier (Tandon and Joshi 1973 and Joshi 1978). Camera Lucida drawings were made of the parasites found in blood slides with precise details and measurements taken.

3. Observations

Histomorphological and morphometric studies made on the species of *Trypanosoma* and *Trypanoplasma* revealed the following characteristics, and accordingly

with the help of existing literature three new species of haemoflagellates/haematozoans are described here :

Parasite : *Trypanosoma aori* (sp. nov.)

Host : *Mystus aor*.

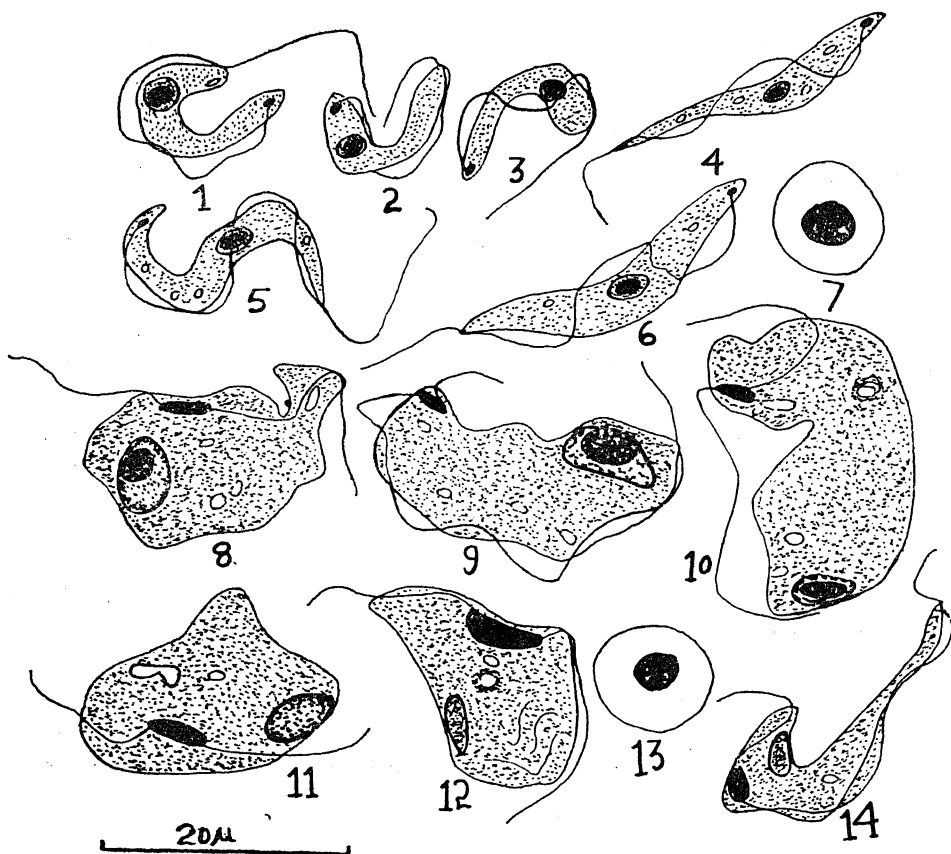
Location : Plasma of the host fish.

Locality : River Gomati, Lucknow.

Diagnosis and descriptions : (figures 1-6) table 1.

Body : Parasites were short (figures 1-3), elongated and partly stumpy. Out of many forms seen in three stained preparations, some typically large elongated forms were also found (figures 4-6). These forms mainly had both ends blunt or rounded, while few forms had pointed or beak shaped extremities.

Figures 7 and 13 are those of the RBC's of the host fishes to give a comparative idea of the blood cell size and the parasite.



Figures 1-14. 1-3. Small sized forms of *T. aori* (sp. nov.). 4-6. Large sized form of *T. aori*. 8, 9, and 10. *T. mysti* (sp. nov.) 7. RBC of *M. aor*, the host fish. 11, 12 and 14. Polymorphic forms of *T. atti* (sp. nov.). 13. RBC of *W. attu*, the host fish.

Cytoplasm : It stained bluish purple, with fine azurophilic dusty granules and granulation appeared denser in the smaller forms, than in the larger ones, which also showed vacuoles. Myonemes were not seen in either of the forms.

Nucleus : It was distinct in all the forms, situated towards either of the two extremities in smaller forms (figures 1-3) or almost in the centre (figures 4-6). In most of the forms, it was rounded or oval, with a distinct karyosome. Karyosome contained, more dense and hyperbasophilic contents than the surrounding karyoplasm.

Kinetoplast : It was present almost at the posterior terminal end and was rounded or slightly elongated.

Flagellum : It always arises from the inner end of the kinetoplast, runs towards the anterior end bordering the undulating membrane before being free at the anterior extremity. In most cases it took light basophilic stain. The free flagellum showed much variation in size.

Undulating membrane : This structure was conspicuously present in all elongated forms and was well differentiated from the body.

Remark : A distinct polymorphism existed, with high parasitemia and low instance of infection.

Parasite : *Trypanoplasma mysti* (sp. nov.)

Host : *Mystus aor*.

Location : Plasma of the host fish.

Locality : River Gomati, Lucknow.

Diagnosis and description : (figures 8, 9 and 10) table 2.

Body : The trypanoplasmid forms were stoutly elongated and irregularly curved (figures 8, 9 and 10). All forms showed conspicuous body width (table 1). Both ends of the parasite were blunt and wide.

Cytoplasm : The cytoplasm is densely packed with fine to coarse granules. It took deep bluish black stain. Vacuoles were frequently present and at places were dense, surrounded by cytoplasmic granules (figure 10).

Nucleus : There occurred marked variation in the shape, size and position of the nucleus of this new species of *Trypanoplasma*. In few forms it was mid-anterior (figure 8) and parallel to kinetoplast, while in others it was situated at either of the two ends (figures 9, 10), being quite away from the kinetoplast. The nucleus always showed a distinct and deeply stained karyosome, which occupied much space within the nucleus. Nuclear chromatin around the karyosome was thinly scattered. The karyosome always took a deep bluish black stain, whereas the nuclear chromatin was purple blue. The nuclear shape varied from ovoid (figure 8), reniform (figure 9) to drop shaped (figure 10).

Kinetoplast : Like that of the nucleus, it also revealed conspicuous variations in the shape, size and position. It was rod shaped in few forms (figures 8, 9) and rounded (figure 10) in others. It is situated either at extreme terminal end

Table 1. Morphometric comparison of *T. aori* (sp. nov.) with other trypanosomes described from the freshwater siluroide hos from the Indian subcontinent.

Particulars	Species	<i>T. bairachi</i> (Qadri 1962)	<i>T. sacco-branchi</i>	<i>T. Mukundi</i> (Raychoudhuri and Mishra 1973)	<i>T. baigulensis</i> (Pandey and Pantley 1978)	<i>T. naguri</i> (Tandon and Joshi 1973)	<i>T. vittati</i> (Joshi 1976)	<i>T. seenghali</i> (Mandal 1980)	<i>T. tandoni</i> (Small form)	<i>T. aori</i> (sp. nov.) (Long form)
1. Length of free flagellum		9-14*	11-15	4-6.5 ¹	4-4.5 ¹	4.2-21.0	6.5-14.0	8.5** (4.0-12.0)	14.5 (11.5-18.5)	18.2 (15.0-19.6) (10-12.5)
2. Length of body		22-29	38-47	9-15 ² 18-23.5 24-28.5	5-5.6 ² 10.2-12.3 18.1-20.5	8.2-35.3	18.2-34.8	24.5 (17.8-28.0)	23.5 (20.0-25.0)	17.6 (14.8-20.2) (29.5-30.0)
3. Total body length		31-33	38-47	22-28.5 32.5-43.5	12.1-14.5 20.3-22.5	26.1-53.8	29.7-47.4	33.0 (27.5-38.0)	38.0 —	35.8 (30.0-39.8) (43.6)
4. Width of body at the centre of nucleus		1.5-3.5	1-1.5	1.9-2.5 2.0-2.5	2-2.1 1.8-2.5	1.1-3.0	1.3-2.3	1.71 (1.2-2.5)	1.5 (1.3-1.8)	3.0 (2.6-4.2) (4.0-5.2)
5. Width of undulating membrane		×	×	×	×	0.2-1.8	0.6-1.4	1.4 (0.9-2.6)	0.6 (0.3-0.95)	1.4 (1.0-2.5) (1.2-2.8)
6. Long axis of the nucleus		2-3	2.75-3.2	2.25-3.75 2.25-3.75	1.1-1.6 1.0-1.5	1.2-5.0	1.9-4.0	2.75 (1.5-3.5)	2.5 (2.25-3.0)	1.9 (1.3-3.2) (2.2-4.4)
7. Short axis of the nucleus		1-1.5	0.7-1.0	1.75-2.25 1.75-2.25	1.2-1.5 1.7-2.0	0.7-1.6	0.7-1.8	1.7 (1.2-2.3)	0.75 (0.5-1.0)	1.5 (1.0-2.5) (0.9-2.2)

8. Distance of nucleus from a flagellar end	×	×	×	×	×	×	6-8 7-10	3.2-15.6	8.0-14.0	11.4 (8.5-15.0)	10.0 (9-11)	5.1 (3.2-11.0) 1.6 (0.6-2.0)	12.0 (9.0-14.2) 1.2 (0.9-1.5)
9. Length of karyosome	×	×	×	×	×	×	×	×	×	×	×	×	×
10. Width of karyosome	×	×	×	×	×	×	×	×	×	×	×	1.2 (0.9-1.2)	0.8 (0.6-1.2)
11. Diameter of kinetoplast	1.1	0.75-1.0	0.75	×	×	×	×	×	×	×	×
12. Length of kinetoplast	×	×	×	×	×	×	×	0.4-1.6	0.9-1.7	0.9 (0.3-1.2)	1.5 (1.0-1.75)	0.9 (0.6-1.2)	1.0 (0.6-1.3)
13. Width of kinetoplast	×	×	×	×	×	×	0.2-0.6	0.3-1.8	0.8-1.8	0.5 (0.3-1.2)	0.76 (0.5-1.0)	0.65 (0.4-0.9)	0.4 (0.2-0.9)
14. Post kinetoplast distance	×	×	×	×	×	×	0.2-0.8	0-1.2	1.1-2.0	1.20 (0.4-2.0)	1.5 (0.5-2.0)	1.1 (0.7-1.3)	1.4 (0.8-1.8)

Values are quoted as presented by their authors. These are either ranges* or mean and range**. Numbers 1 and 2 denote the size of the polymorphic forms. All measurements are in microns. (×) cross signs denote the values not given by the author.

Table 2. Comparative morphometric characteristics of the new species and allied species of the *Trypanoplasma* (Cryptobia).

Particulars	Species Author	<i>C. borrelli</i> (Meshil 1901)	<i>C. salmositica</i> (Katz 1951)	<i>C. catartactai</i> (Putz 1972)	<i>C. indica</i> (Mandal 1979)	<i>T. mysti</i> (sps. nov.)	<i>T. atti</i> (sps. nov.) longform	Ovoid form
Length of anterior flagellum		×	16.5	(9.6-13.2)	25.0 (21.0-30.0)	13.8 (10.0-22.0)	9.6 (7.5-12.0)	7.5 (6.0-9.0)
Length of posterior flagellum		×	8.96	×	10.5 (9.0-12.0)	11.2 (8.0-15.0)	8.3 (7.0-10.5)	9.0 (6.0-15.0)
Body length		20-25	14.9	17.0 (14.17-18.9)	28.5 (25.0-30.5)	28.2 (24.0-34.2)	35.3 (26.0-50.0)	22.2 (18.0-27.0)
Body width		3-4	2.0-9.6	2.0 (1.5-2.2)	8.5 (6.0-10.5)	10.9 (8.8-13.0)	6.8 (5.0-8.0)	12.0 (9.0-14.0)
Length of the nucleus		×	3.5	×	7.5	6.5 (5.0-8.0)	5.5 (3.5-7.0)	4.9 (4.0-6.0)
Width of the nucleus		×	1.5	×	5.0	3.4 (2.3-4.0)	1.9 (1.0-3.0)	2.9 (1.5-3.5)
Length of the kinetoplast		×	4.58	5-6	5.0	1.3 (0.5-2.5)	4.0 (3.0-5.0)	5.5 (4.5-9.4)
Width of the kinetoplast		×	0.78	0.75-2.0	1.5	0.9 (0.4-1.5)	1.4 (0.9-2.0)	2.5 (1.8-3.7)
Length of karyosome		×	×	×	×	3.0 (2.2-4.5)	—	—
Width of karyosome		×	×	×	×	3.0 (1.0-3.2)	—	—

All values are in microns and are mean and range: Sign of cross (×) denotes the value not given by the author and a dash (—) means absence of the organoid.

Note: All measurements for the new species of *Trypanoplasma*, described here, are for 7 observations each.

(figure 9) or nearly towards the central periphery of the cell. It always stained deep bluish black.

Flagellum : All the forms possessed two free flagella, which arise from the two ends of the same kinetoplast. Anterior flagellum was usually larger than the posterior (table 2). The posterior flagellum either runs through the cytosome (figure 8) or borders the undulating membrane (figure 9), before being freed at the post extremity.

Undulating membrane : It was not seen in few forms (figure 8), but distinctly present in others (figures 9, 10).

Remark : This newly described species of *Trypanoplasma aori* was found harbouring the same specimen of the host species, *M. aor*, to which the *T. aori* (sp. nov.) harboured. The host species thus showed a multispecies parasitemia of high intensity. Only one specimen of the host fish, out of 40 observed was found to be parasitized by these haematozoans (Joshi 1979).

Parasite : *Trypanoplasma atti* (sp. nov.)

Host : *Wallago attu*

Location : Plasma of the host fish

Locality : River Gomati, Lucknow.

Diagnosis and descriptions : (figures 11, 12 and 14).

Body : The unicellular body showed marked variations, with characteristic undulations (figure 14), while few others were ovo-triangular (figures 11, 12), with irregular shapes, hence conspicuous differences were noted in body size (table 2).

Cytoplasm : It was homogeneous and densely filled with coarse cytoplasmic granules. The cytoplasmic contents took most hyperbasophilic stain than in any other form described. Vacuoles were present in almost all forms, besides myonemic striations were also seen in few forms (figure 12).

Nucleus : It was short, cylindrical or rod like (figures 11, 12), reniform or ovoid (figure 14). Karyosome was not seen in any form of this species. Nuclear chromatin loosely distributed within the karyoplasm which stained deep purple blue (figures 11, 12, 14) to light reddish purple.

Kinetoplast : It was larger in size than in *T. mysti* (sp. nov.) described above (table 2), and was usually situated towards either of the extremities within the cell (figures 11, 14). It always stained bluish black.

Flagellum : One anterior and one posterior flagella were always present in all forms. In few forms anterior flagellum was longer (figures 11, 12) while in others, posterior (figure 14). In some forms both the flagella free abruptly, after traversing through the cytosome (figures 11 and 14), while in others it borders the outer margin of the cell or the undulating membrane (figure 12) and then frees.

Undulating membrane : It was present in few forms (figure 14) and not distinct in others (figures 11, 12).

Remark: Two specimens of the host fish out of 65 observed were found harbouring this parasite.

4. Discussion

Despite the fact that the above description is characteristic to the new species of the haematozoans described, the problem of new speciation remains complicated for the haemoflagellates from fish, as also encountered earlier by various authors (Baker 1960; Becker 1970; Putz 1972; Joshi 1978 and Mandal 1979). The problem becomes more complicated when a particular species of these haematozoan parasites show a great degree of polymorphism (Laired 1948, 1951; Tandon and Joshi 1973 and Joshi 1978). Besides, experimental studies have also revealed that many of these haematozoans are euryhostpatalic (Becker 1977). Further, recently Froes *et al* (1978, 1979) and Grogl *et al* (1980) have described six and one new species of the trypanosomes, respectively, from seven new host fish. They have also used the same criteria of species specificity and varying morphometric characteristics in all cases to create new speciation.

In the present case *T. aori* (sp. nov.) is not only different in having a new host fish, hitherto not described, but also in various cytomorphological and meristic characteristics. A high degree of polymorphism was also evident. The morphometric differences from the twentytwo species of the trypanosomes described earlier from the Indian freshwater teleosts viz, four species by Qadri (1955, 1962), one by Hasan and Qasim (1962), one by Misra *et al* (1973), two by Tandon and Joshi (1973), two by Ray-Chaudhury and Misra (1973), one by Pandey and Pandey (1964), seven by Mandal (1975, 1977, 1978 and 1979) and four by Joshi (1976 and 1978). The new species, *T. aori* is also conspicuously different from many other forms described from various freshwater host species by Dutton *et al* (1906), Hoare (1932), Baker (1960), Smirnova (1970) and Abolarin (1970). The degree of polymorphism encountered here is well comparable with those described by Laired (1952) for several species of trypanosomes.

Table 1 provides a comparative account of meristic morphological characteristics of eight species of trypanosome described from the siluroid hosts inhabiting the freshwater realms of the Indian subcontinent. Interestingly, when linear overlaps of all these species including the new species described here, are compared (as suggested by Mayr 1969), then at least 10–30% of all the measurements given for a species are overlapped by one or the others. However, despite these facts, the present new species *T. aori*, described here, have two major differences than the others described earlier. These are (i) small forms are most characteristic in appearance (acquiring a twisted grub-like structure) and (ii) these forms have usually maximum body width at the centre of the nucleus. Besides, the flagellar length, post nuclear distance, presence of the karyosome within the nucleus and comparatively blunt posterior extremity are its species characteristics.

Two new species of trypanoplasma viz., *T. mysti* and *T. atti*, differed not only from *C. indica* (Mandal 1979), described from *M. vittatus*, but also from related species like *C. borsoli* (Laveran and Mesnil 1901), *C. salmositica* (Katz 1951) and *C. cataractae* (Putz 1972), in almost all morphometric and cytomorphic character. It is important to mention that till recently the diflagellate haematozoans

harbouring in the blood stream of the fishes were described under the genus *Cryptobia* (viz., Katz 1951) and since recently it has been resolved that all the diflagellate haematozoans be described under the generic name of *Trypanoplasma*, as pointed out by Woo (1979).

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References

- Abolarin M O 1970 A note on the trypanosomes of the African freshwater fishes and some comments on the possible relationship between taxonomy and pathology in trypanosomes; *Bull. Epizoot. Dis. Afr.* 18 221-228
- Baker J R 1960 Trypanosomes and dactylosomes from the blood of freshwater fish in East Africa; *Parasitol.* 5 515-526
- Becket C D 1970 Haematozoa of fishes, with emphasis on North American Records. Spl. Publ. No. 50 82-100 American Fish Society, Washington, D. C.
- Becker C D 1977 Flagellate parasites of fish; *Parasitic Protozoa* (ed) J P Kreier Academic Press pp 357-416
- De-Mello I F and Valles C F 1936 On the Trypanosoma found in the blood of the Indian freshwater fish *Clarias batrachus* (Linn.); *Proc. Indian Acad. Sci.* B38 120-124
- Dutton J E, Tood J L and Tobey E N 1906 Concerning haemoflagellates of an African fish *Clarias angelensis*; *J. Med. Res.* 15 491-494
- Froes O M, Fortes E, Lima D F and Leite V R V 1978 Trypanosomes (Protozoa, Kinetoplastida) of freshwater fishes from Brazil I. Description of three new species; *Rev. Bras. Biol.* 38 461-468
- Froes O M, Fortes E, Lima D F and Leite V R V 1979 Trypanosomes (Protozoa, Kinetoplastida) of freshwater fishes from Brazil. II. New trypanosomes from "Casudos" (Pisces Loricaridae); *Rev. Bras. Biol.* 39-425-429
- Grogl M, Marinkelle C J M F, Sanchez N and Guhl F 1980 *Trypanosome magdalenae* sp. n (Protozoa: Kinetoplastida) from freshwater teleosts, *Patenia kraussi*; *Colombia J. Parasitol.* 66 1022-1026
- Hasan R and Qasim S Z 1962 *Trypanosoma punctati* n.sp. from the fish *C. punctatus* common freshwater murrel of India; *Z. Parasitenkd.* 22 118-122
- Hoare C A 1932 On protozoan blood parasites collected in Uganda; *Parasitol.* 24 210-217
- Joshi B D 1976 On two new species of trypanosomes from two fresh water teleosts; *Indian J. Zootomy* 17 5-10
- Joshi B D 1978 Two new species of trypanosomes from freshwater teleosts; *J. Anim. Morphol. Physiol.* 25 1-7
- Joshi B D 1979 On the occurrence of trypanosomes in the blood of some freshwater teleosts of Lucknow (UP), India; *Proc. Indian Acad. Sci.* B88 59-63
- Katz M 1951 Two new haemoflagellates (*Gen. Cryptobia*) from some Western Washington; *J. Parasitol.* 36 245-250
- Laired M 1948 *Trypanosoma heptatrete* sp. nov. a blood parasite of hagfish; *Nature (London)* 61 440-441
- Laired M 1951 Some trypanosomes of New Zealand fish; *Proc. Zool. Soc. London* 12 285-309
- Lavran A and Mesnil F 1901 On the flagellates with undulating membrane of the fishes (*Gen. Trypanosoma* gruby and *Trypanoplasma* n.gen.); *C.R. Acad. Sci.* 133 670-675
- Lingard A 1904 A short account of the various *Trypanosomata* found to date in the blood of some of the lower animals and fish; *Indian Med. Gaz.* 49-46-449

Heidenhain's Azan and Delafield's haematoxylin/eosin techniques were used for routine histological studies. For histochemical studies the following techniques mainly from Pearse (1968) have been employed. (1) Periodic acid/Schiff (PAS) method of Hotchkiss and McManus, (2) PAS after diastase digestion, (3) PAS after acetylation followed by deacetylation, (4) alcian blue 8 GX (2.5 and 1.0 pH) for acid mucosubstances, (5) mercury bromophenol blue method of Mazia *et al*, (6) Millon's reaction after Baker, (7) *p*-dimethyl aminobenzaldehyde nitrite method of Adams, (8) potassium permanganate/AB method of Arvy and Gabe, (9) ferric ferricyanide method of Chevrement and Frederic, (10) ninhydrin/Schiff method of Yasuma and Itchikawa, (11) Congo red technique for glycoproteins, (12) Sudan black B technique for lipids after Chiffelle and Putt, (13) copper phthalocyanin method of Klüver and Barrera for phospholipids, (14) methyl green/pyronin Y method of Kurnick for nucleic acids, (15) Feulgen reaction of Feulgen and Rossenbeck for DNA.

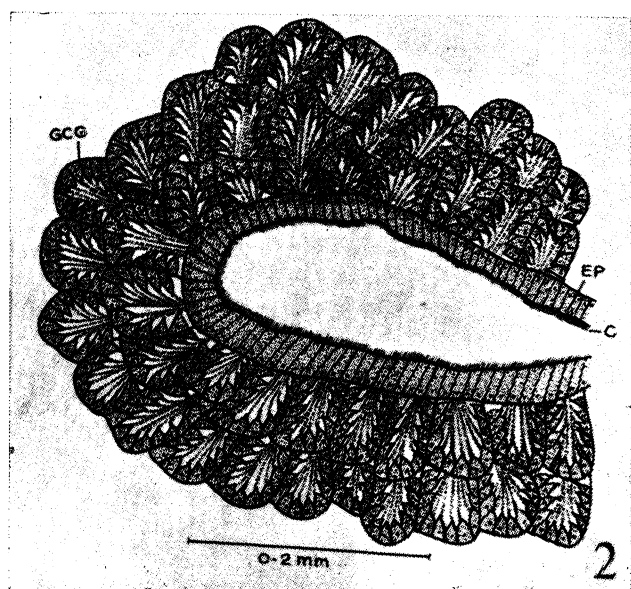
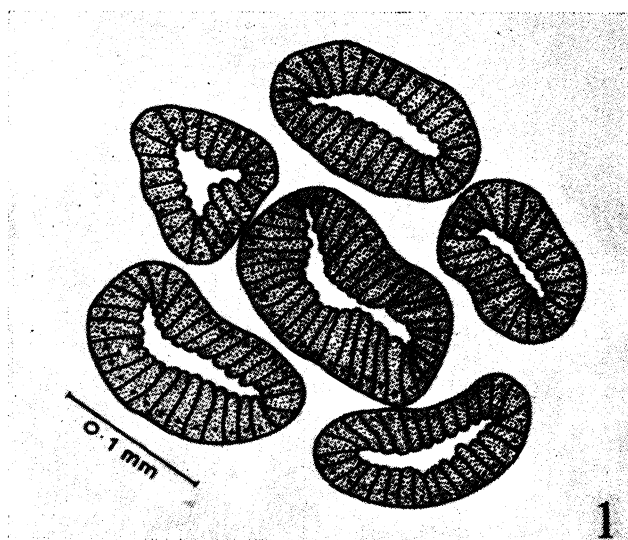
3. Observations

3.1. *Histology*

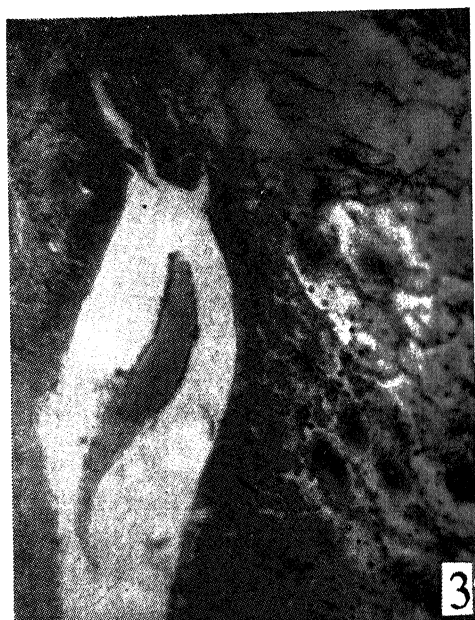
The albumen gland is a white opaque mass lying dorsal and just posterior to the pericardial cavity. In live condition, the albumen gland is creamish white in colour and consists of large number of tubules (figure 1) separated from one another by a thin layer of connective tissue. These tubules are spherical to oval in shape. The wall of each tubule consists of large cuboidal to columnar cells measuring about $58.5\ \mu\text{m}$ in height. Each cell contains a large basal nucleus and is glandular in nature and secretory droplets are seen towards the apex of the cell. The tubules lead into a number of small ducts which unite to form a common duct which in turn opens into the capsular gland. The secretions of the albumen gland are acidophilic as well as basophilic in nature. These secretions are at their peak in the breeding season which is from December to June and the albumen gland is found to be considerably small in the non-breeding season.

The oviduct opens into the albumen gland at a point below the kidney. The albumen gland turns abruptly downwards making an acute angle with the oviduct and it then recoils on itself passing ventrally to open into the capsule gland. The albumen gland opens into the ventral wall of the capsule gland by a short duct which is lined by a columnar ciliated epithelium, interspersed with few mucocytes. It is surrounded by a thick layer of circular muscles which on contraction close the passage between the two folds.

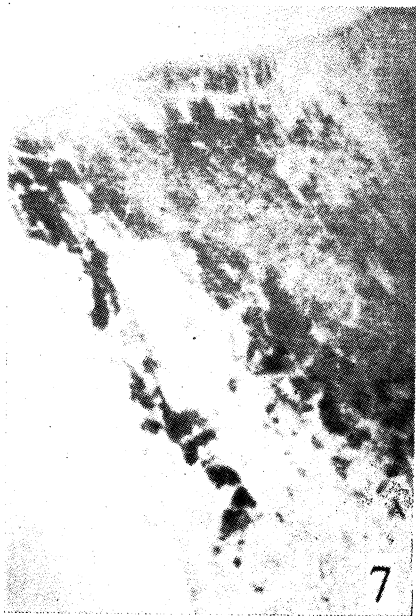
The capsule gland is a creamish yellow glandular mass. In reproductively active individuals this gland attains a thickness of about 2 mm and a length of about 5 mm. The lateral walls are thickened and composed of groups of gland cells lying at various heights (figure 2). They are packed together tightly with a thin layer of connective tissue in between. The ducts of the walls run parallel to one another and open between the columnar ciliated cells lining the lumen. This ciliated epithelium covers the narrow dorsal wall, under which a layer of gland cells is developed (figure 3). The lobes are composed of gland cells filled with small colourless spherules. These spherules stain lightly with iron



Figures 1-2. 1. Schematic representation of the albumen gland. 2. Schematic representation of the capsular gland.



Figures 3–4. 3. Transverse section of the capsular gland showing mucous cells
4. Transverse section of the capsular gland (Azan).



Figures 5-8. 5. Albumen gland tubules showing the PAS reactive secretory droplets. 6. Capsule gland showing PAS reactive substances. 7. Capsular gland showing mucous secretions (AB 2.5 pH). 8. Capsular gland showing proteinaceous secretions (BPP).

haematoxylin and faint blue with alcian blue. After Heidenhain's Azan technique spherules are reddish or orange and others blue. It appears that two types of secretions are produced by these cells. Near the posterior end of the capsule gland two narrow transverse strips of tissue one on either side arise near the opening of the albumen gland. These strips separate the right and left posterior tip from the main mass. These and the posterior tips of the gland are made up of mucous cells. Similar cells are found at the anterior extremity of each lateral lobe (figure 4). Beneath the ciliated epithelium of the strips a circular muscle layer is present. The cells constituting the main part of the gland are filled with large colourless granules. They stain with iron haematoxylin but are negative to alcian blue. With Heidenhain's Azan the granules take a deep red stain, whereas the cytoplasm stains deep blue. The distal tip of each duct is filled with the mucoid substances and no granules are visible. Two types of secretions are produced, a mucoid substance and a protein.

3.2. Histochemistry

Both the albumen gland and the capsular gland are intensely positive to PAS technique (figures 5 and 6). This PAS reactivity was resistant to saliva treatment suggesting the absence of glycogen. This reactivity was abolished after acetylation and was restored after deacetylation indicating the presence of 1:2 glyco-groups. They showed a moderate positivity to Schiff's reagent without prior oxidation. Those cells lining the posterior tips and the anterior extremity of each lateral lobe of the capsular gland showed positivity to alcian blue at 1.0 and 2.5 pH (figure 6), whereas the albumen gland showed a negative response.

Both the gland cells were positive to mercury bromophenol blue, a technique for basic proteins. Thus the proteinaceous nature of the gland cells is indicated (figure 7). This was further confirmed by subjecting the slides to deamination with Vanslyke's reagent. When subjected to *p*-DMAB nitrite method and Millon's reaction a negative response was observed. With ninhydrin/Schiff and chloraniline T/Schiff the albumen gland showed an intense positivity whereas the capsule gland showed a moderate positivity, thus suggesting the presence of large and moderate quantities of protein bound NH_2 groups respectively. The presence of disulphides was indicated by their response to KMnO_4/AB technique. The albumen gland showed an intense reaction to ferric ferricyanide for S-H groups, while the capsule gland stained faintly.

There is no considerable quantity of lipid as suggested by Sudan black B technique, but copious volumes of phospholipids are present as evidenced by a very strong positivity to copper phthalocyanin.

The presence of nucleic acids such as RNA and DNA were traced by methyl green/pyronin Y reaction and Feulgen reaction respectively.

From the ensemble of these reactions it could be stated that both the albumen gland and capsule gland are highly proteinaceous. They seem to contain large quantities of basic protein, cystine, sulfhydryls, amino bound proteins, carbohydrates, lipids and phospholipids. The secretions of the albumen gland are rich in carbohydrates and protein whereas that of the capsule gland is a mucoprotein. Results of the above histochemical reactions are presented in table 1.

Table 1. Histochemical reactions of the albumen and capsular glands of *Thais bufo*.

Histochemical test applied	Reacting group	Results	
		Albumen gland	Capsular gland
Periodic acid/Schiff (PAS)	Carbohydrate	+++	+++
PAS/saliva	Glycogen	+++	+++
Acetylation/PAS	1 : 2 glycols	—	—
Deacetylation/PAS	1 : 2 glycols	++	++
Schiff's without prior oxidation	Aldehydes	++	++
AB 2.5 pH	Mucins	—	+++
AB 1.0 pH	Mucins	—	+++
Mercury bromophenol blue	Basic protein	++	+++
Millon's reaction	Cystine	—	—
DMAB nitrite method	Tryptophan	—	—
Ninhydrin/Schiff	NH ₂ groups	+++	++
Permanganate/AB	Disulphides	++	++
Ferric ferricyanide	Sulphydryls	+++	+
Sudan black B	Lipids	++	++
Copper phthalocyanin	Phospholipids	+++	+++
Methyl green/pyronin Y	Nucleic acid	++	++
Feulgen reaction	DNA	++	++

+++ = Intensely positive; ++ = Moderately positive; + = Faintly positive; — = Negative.

4. Discussion

The albumen gland is composed of a large number of tubules which are lined by secretory cells. A change in its secretory activity was noticed with season. The secretions of the albumen gland seem to contain PAS positive granules without glycogen, protein and phospholipids. The eggs as they pass through the albumen gland are bathed by the albuminous secretions of the gland cells. These secretions are helpful in nourishing the embryos. The fact that the secretions of the albumen gland contain the polysaccharide galactogen rather than glycogen was established by the studies of May (1934) and Baldwin and Bell (1938) in the snail *Helix pomatia*. Fantin and Vigo (1968) reported the presence of galactogen and protein in the secretions of the albumen gland of *L. stagnalis*. Plesch et al (1971) observed PAS positive secretory droplets in the albumen gland of *L. stagnalis*.

The capsule gland in *T. bufo* is large and attains a thickness of about 2 μ m, when the animals are in the active reproductive phase. As the eggs pass down the albumen gland into the capsule gland along with the albuminous secretion

a capsule is formed around a group of eggs along with the albumen. All the gland cells of the capsule gland, except those of the posterior tip and anterior border of each lobe, produce a double secretion. By the intervention of these two secretions the fibrous wall of the egg capsule is produced. The capsule is thus mucoprotein in nature.

Studies on the capsule gland in particular are meagre. This gland secreting the egg capsule is present in some prosobranchs and the capsule is finally hardened in the pedal gland in Cypraeacea, Lamellariaceae and in most Stenoglossans. The capsule gland is absent in *Onchidella* and Pulmonates (Fretter 1943). The mucoprotein secretions of the capsular gland in *T. bufo* aid in the formation of the fibrous wall of the capsule.

Acknowledgements

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References

- Baldwin E and Bell D J 1938 Preliminary investigation of galactogen from the albumen gland of *Helix pomatia*; *J. Chem. Soc.* 1938 1461-1465.
- Fantin B A M and Vigo E 1968 Histochemistry of the glands associated with the reproductive tract of *Lymnaea stagnalis*; *Histochemie* **15** 303-311
- Fretter V 1941 The genital ducts of some British stenoglossan prosobranchs; *J. Mar. Biol. Assoc. U.K.* **25** 173-211
- Fretter V 1943 Studies on the functional morphology and embryology of *Onchidella celtica* (Forbes and Hanley) and their bearing on its relationships; *J. Mar. Biol. Assoc. U.K.* **25** 635-730
- Fretter V 1946 The genital ducts of *Theodoxus*, *Lamellaria* and *Trivia* and a discussion on their evolution in the prosobranchs; *J. Mar. Biol. Assoc. U.K.* **26** 312-351
- Fretter V and Graham A 1962 British prosobranch molluscs; *Roy. Soc. Lond.* 144 series pp. 755
- Kugler O E 1965 A morphological and histochemical study of the reproductive system of the slug, *Phyllomycus carolinianus* (Bosc); *J. Morphol.* **116** 117-132
- May F 1934 Chemische und biologische untersuchungen uber Galactogen; *Ztschr. f. Biol.* **95** 287-297
- Pearse A G E 1968 *Histochemistry: Theoretical and applied* (London: Churchill) Vol. **1**
- Plesch B, Marijke de J B and Boer H H 1971 Histological and histochemical observations on the reproductive tract of the hermaphrodite pond snail *Lymnaea stagnalis* (L.); *Neth. J. Zool.* **21** 180-201
- Rangarao K 1963 The polysaccharides of the reproductive system of the land snail *Ariophanta ligulata* in the formation of egg capsules; *J. Anim. Morphol. Physiol.* **10** 158-163

Effect of temperature on food intake, growth and conversion efficiency of *Eupterote mollifera* (Insecta: Lepidoptera)

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Abstract. The effect of temperature on food intake, growth and conversion efficiency has been studied in the final instar male and female larvae of *Eupterote mollifera*. Food consumed, assimilated and metabolised decreased with increase in temperature. The larval duration decreased from 12 days for the group reared at 22° C to 5 days for the group reared at 37° C. While the rates of feeding, assimilation and conversion increased with increase in temperature, high conversion efficiencies (K_1 and K_2) were observed for the larvae reared at 27 and 32° C.

Keywords. Temperature ; food intake ; *Eupterote mollifera*.

1. Introduction

Many species of lepidopterous larvae are known to cause serious damage to economically important plants (Ayyar 1963). While the energetics of food utilization in relation to temperature have been reported for a few lepidopterans (Waldbauer 1968 ; Mathavan and Pandian 1975 ; Pitchairaj *et al* 1977), there are no such studies on *Eupterote mollifera* which is a common pest on drum-stick plant. This paper, based on the earlier studies of energy intake and expenditure pattern (see Palanichamy *et al* 1979), reports the effect of temperature on food utilization in the tropical moth *E. mollifera*.

Lepidopterous larvae consume more than 70% of the total food intake during final instar (Waldbauer 1968 ; Mathavan and Pandian 1975) and accumulate sufficient energy (Delvi and Pandian 1972 ; Pandian 1973) to tide over the non-feeding pupal stage. Palanichamy *et al* (1979) reported that the final instar larvae of *Eupterote mollifera* consumed 71.4% of the total food intake at $30 \pm 2^\circ \text{C}$. Hence, the effect of temperature on food utilization has been studied only in the final fifth instar larvae of *E. mollifera*.

2. Materials and methods

Newly hatched first instar larvae of *Eupterote mollifera* were collected from the field and reared as a group in 8 litre glass trough. As soon as the larvae entered the final instar, the males and females were separated out, weighed and reared individually in 1 litre glass container at four different temperatures (22, 27, 32 and 37° C) with an accuracy of 1° C. Sex identification was confirmed after adult emergence and any larvae identified wrongly was discarded from the experiment. The larvae were fed *ad libitum* with fresh leaves of *Moringa pterygosperma* (drum-stick plant) daily throughout the experimental period. Daily food intake was measured by a standard gravimetric method (Waldbauer 1968) with all weighings accurate to 0.01 mg. Food, faeces and larvae were dried overnight at $90 \pm 2^\circ \text{C}$ to constant weight for purposes of calculations (see Palanichamy *et al* 1979).

3. Results

3.1. Larval duration and growth

The changes in the instar duration, live body weight and growth in relation to four different temperatures are indicated in table 1. While there were distinct differences in the live body weight of male and female larvae at all temperatures, least differences were observed between the two sexes reared at different temperatures. However, the instar duration decreased from 12 days for the larvae reared at 22° C to 5 days for the larvae reared at 37° C. While the maximum weight of male (710 mg) and female (916 mg) larvae was observed when reared at 22° C, highest growth was observed (male : 124 ; female : 164 mg) for the larvae reared at 32° C.

Table 1. Initial and final live weight of fifth instar larvae of male and female *Eupterote mollifera* fed on the leaves of drum-stick plant *Moringa pterygosperma* at different temperatures.

Temperature (° C)	Fifth instar duration (days)	Initial weight (mg)		Final weight (mg)		Growth (mg)	
		Male	Female	Male	Female	Male	Female
22	12.0 \pm 1.00	229 \pm 36.2	253 \pm 32.6	710 \pm 90.4	916 \pm 84.5	101 \pm 10.9	134 \pm 14.2
27	7.5 \pm 0.50	227 \pm 39.6	240 \pm 38.9	649 \pm 82.2	855 \pm 94.7	103 \pm 12.1	142 \pm 9.7
32	7.7.0 \pm 0.00	216 \pm 32.8	231 \pm 27.4	691 \pm 88.1	748 \pm 92.9	124 \pm 11.3	164 \pm 8.6
37	5.0 \pm 1.00	151 \pm 29.7	167 \pm 24.1	562 \pm 79.3	601 \pm 77.9	82 \pm 9.0	87 \pm 8.1

3.2. Food utilization

The amount of food consumed and assimilated were high for either sexes of larvae reared at 22° C and least for those reared at 37° C (figure 1). However, the values remained similar for males and females reared at 27 or 32° C. In spite of higher food consumption and assimilation for the larvae reared at 22° C, maximum growth (male : 124 ; female : 164 mg) was observed for the larvae reared at 32° C. These values are higher than those reported for the same species (male : 107.7 ; female : 148.5 mg) reared at $30 \pm 2^\circ \text{C}$ (see Palanichamy *et al* 1979).

3.3. Rates of bioenergetics of feeding

At all the temperatures tested, the rates of bioenergetics of feeding did not vary much between the male and female larvae (figure 2). While the rates of bio-

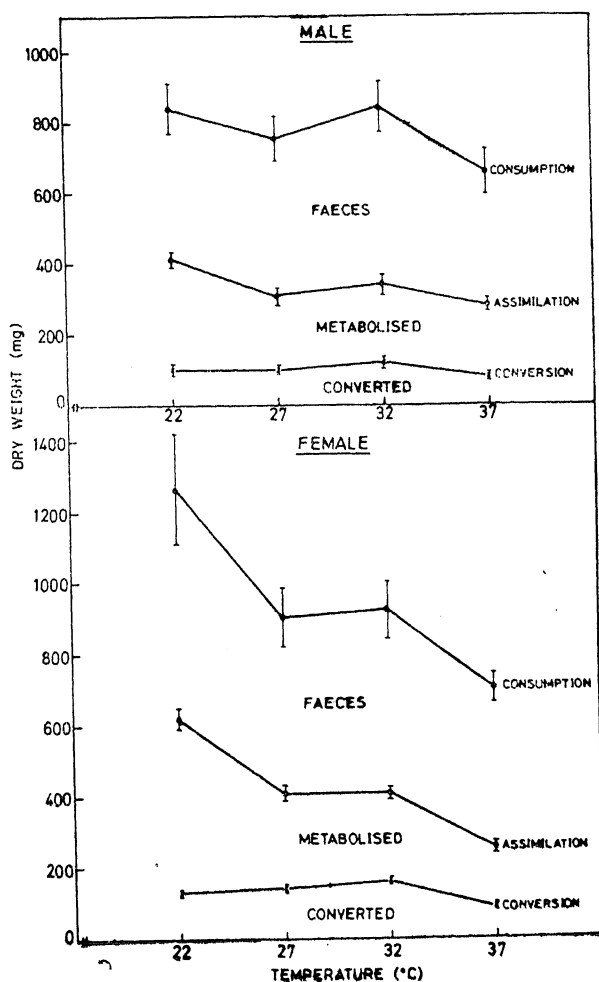


Figure 1. Effect of temperature on food intake, assimilation and conversion in fifth instar larvae of male and female *Eupterote mollifera* fed on the leaves of drum-stick plant *Moringa pterygosperma*. Each value represents an average of 10 larvae ($= \pm \text{S.D.}$).

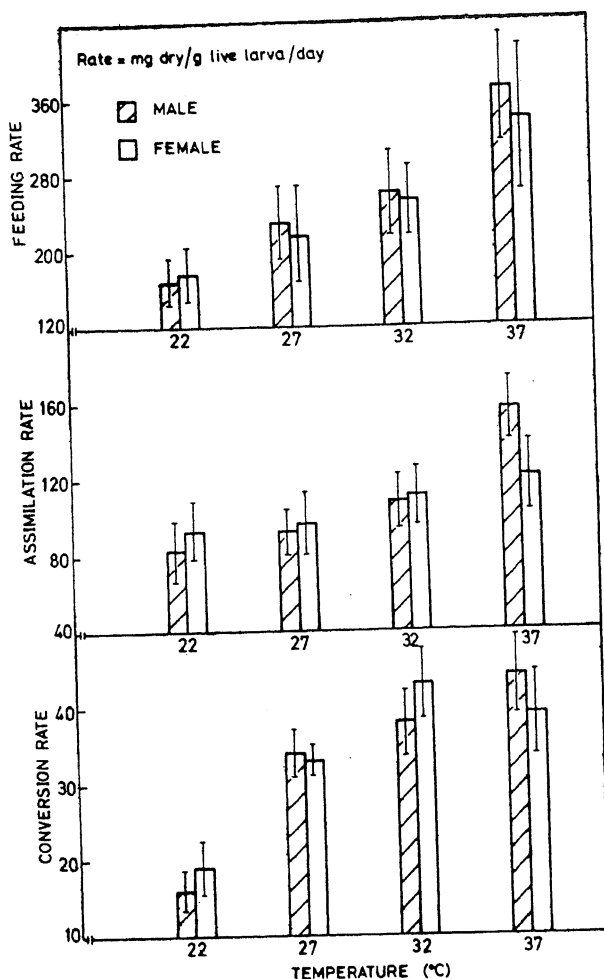


Figure 2. Histogram represents the effect of temperature on the rates of feeding, assimilation and conversion in male and female of fifth instar larvae *Eupierote mollifera* fed on the leaves of drum-stick plant. Each value represents an average of 10 larvae (\pm S.D.).

energetics of feeding indicated gradual increase with increases in temperature, the conversion rate did not show much variation for the larvae reared at 32 and 37° C. However, distinct differences were observed in conversion rate for the larvae reared at 22 and 27° C.

3.4. Assimilation and conversion efficiencies

The changes in the assimilation and gross and net conversion efficiencies are represented in figure 3. Assimilation efficiency decreased for either sexes with increase in temperature (male : 50 ; female : 49% at 22° C to male : 36 ; female : 31% at 37° C). However, high gross (K_1 : %) and net (K_2 : %)

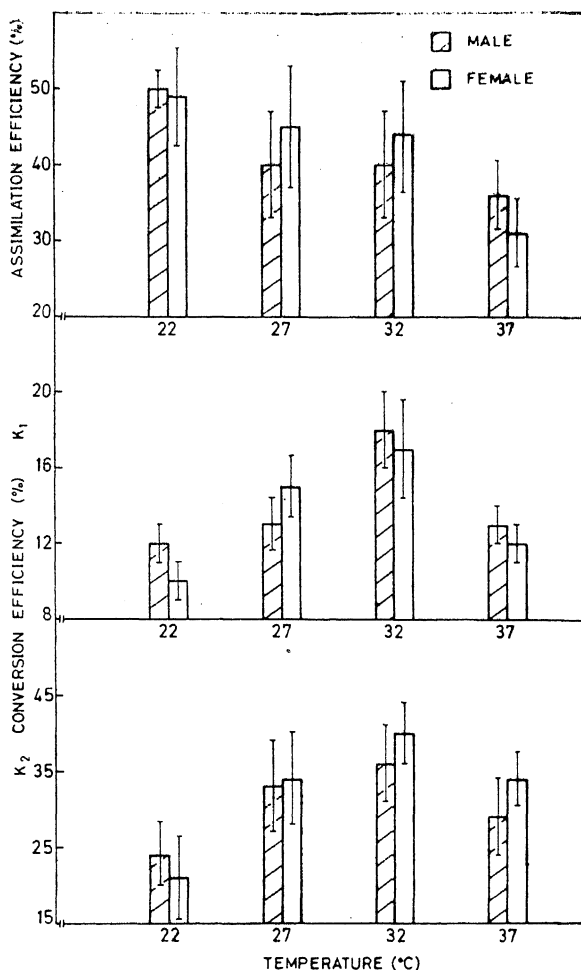


Figure 3. Histogram represents the effect of temperature on the assimilation and conversion (K_1 and K_2) efficiencies in male and female of fifth instar larvae *Eupterote mollifera* fed on the leaves of drum-stick plant. Each value represents an average of 10 larvae (\pm S.D.).

conversion efficiencies were found for either sexes reared at 32° C. As observed in the rates of bioenergetics of feeding, assimilation and conversion efficiencies remained similar for both the sexes at all the temperatures tested.

4. Discussion

The final body weight of fifth instar female *Eupterote mollifera* showed an increase of 51% at 22° C as against the larvae reared at 37° C ; whereas the increase in weight was only 32% for the fifth instar female *Danaus chrysippus* reared at 19° C as against the larvae reared at 37° C (see Mathavan and Pandian 1975). Probably the variation in temperature (22 and 19° C) provided for these two

species may account for the difference. This information supports the fact that many species attain larger final body size in the cooler parts of their distribution (Kinne 1970) and explains the variation in the maximum weights of different ecotypes of insects with seasonal and geographical distribution (Odum 1971).

Food consumed, assimilated, converted and metabolized by *Eupterote mollifera* showed distinct differences between the larvae reared at 22 and 37° C. However, the values did not exhibit distinct differences for the larvae reared at 27 and 32° C. This is in conformity with the findings reported for the lepidopteran *Danaus chrysippus* (Mathavan and Pandian 1975). The high conversion observed for the larvae reared at 27 and 32° C indicates that these temperature ranges are optimum to elaborate best growth.

On an average, the larvae of *Eupterote mollifera* showed nearly 1½ times higher rates of feeding (353 mg/g live larvae/day) and assimilation (138 mg/g live larvae/day) at 37° C than those reared at 22° C. However, this temperature proves to be lethal for the larvae as evidenced by high mortality. The mortality was observed due to reduction in the thickness of the skin which in turn changes the colour from dark brown to reddish brown. The larvae reared at 37° C consumed less food (680 mg) and assimilated with 33.5% efficiency. Thus the assimilated food which are available for metabolic and growth processes is not proportionally increased. This explains the finite body size of the larvae reared at 37° C though the net and gross conversion efficiencies were more than those reared at 22° C.

References

- Ayyar T V R 1963 *Handbook of economic entomology* (Govt. of Madras Publications) pp. 249-250
- Delvi M R and Pandian T J 1972 Rates of feeding and assimilation in the grasshopper *Poecillocerus pictus*; *J. Insect Physiol.* **18** 1829-1843
- Kinne O 1970 Temperature in animals. In *Marine ecology* (ed) O Kinne (London: Wiley Interscience) pp. 407-514
- Mathavan S and Pandian T J 1975 Effect of temperature on food utilization in the monarch butterfly *Danaus chrysippus*; *Oikos* **26** 60-64
- Odum E P 1971 *Fundamentals of ecology* (Saunders Co.) pp. 574
- Palanichamy S, Thangaraj T and Ponnuchamy R 1979 Studies on food utilization by *Eupterote mollifera*; *The Indian Zoologist* **3** 89-92
- Pandian T J 1973 Food intake and energy expenditure patterns in two insect primary consumers; *Curr. Sci.* **42** 423-425
- Pitchairaj R, Mathavan S and Muthukrishnan J 1977 Observations on the effect of temperature on food utilization in a pierid butterfly; *Comp. Physiol. Ecol.* **2** 48-50
- Waldbauer G P 1968 The consumption and utilization of food by insects; *Adv. Insect Physiol.* **5** 229-288

Seasonal variations in the phosphorus contents of the muscle of catfish *Clarias batrachus* L.

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Abstract. Seasonal variations were observed in total acid soluble phosphorus, inorganic phosphorus and phospholipid in the muscle of *C. batrachus* L. The maximum concentration of these constituents were recorded during April, May and June. Thereafter values decreased and the levels remained low during winter months. The observed changes have been correlated with feeding intensity, gonad maturation and spawning. The rise and fall of different phosphorus contents were found to coincide with high and low rate of feeding. There was a gradual rise in the values when gonads advanced towards maturity. The maximum concentration corresponded to the period of peak ripeness (April, May and June). The values declined during the spawning period which possibly indicate the utilization of these reserves for energy. The low phosphorus contents observed in post-spawning and winter appear to be the result of exhaustion of spawning.

Keywords. Seasonal variations ; phosphorus contents ; *C. batrachus*.

1. Introduction

Although phosphorus has been studied in tissues of many fish species (Nakano 1960 ; Nakano and Tsuchiya 1960 ; Chang and Idler 1960 ; Jafri 1965 ; Bhushana Rao 1965), there seems to be no earlier account on the changes in muscle phosphorus contents with season except that in some fish such changes were reported on blood (Shell 1961 ; Siddiqui and Siddiqui 1965 ; Siddiqui and Naseem 1971 ; Siddiqui 1972). In this paper similar observations are being reported in the muscle of catfish *Clarias batrachus* L. a commercially important freshwater fish. In earlier papers seasonal variations in other chemical constituents of this fish have been reported (Bano 1977 ; Bano and Hameed 1979).

2. Materials and methods

Specimens of *C. batrachus* ranging from 18-26 cm in length were procured at monthly intervals from a freshwater pond at Aligarh and maintained alive in a large laboratory aquaria. The fish were left for twenty-four hr for acclimatization before starting the sampling. After that they were removed, killed by decapitation and tissue taken out constantly from anterior trunk region taking

care that only white muscle is removed. The maturity stage of gonad was determined arbitrarily from the scheme suggested by Qayyum and Qasim (1964). The two sexes were analysed separately and it was ensured that the muscle were free of bones. The total acid soluble phosphorus, inorganic phosphorus and phospholipid were determined by the methods described earlier by Bano (1975). Extractions of these fractions were made at cold temperatures.

3. Results and discussion

Monthly values of different phosphorus contents showed a wide range of fluctuation. The mean values are given in the form of annual cycle in figure 1. As is

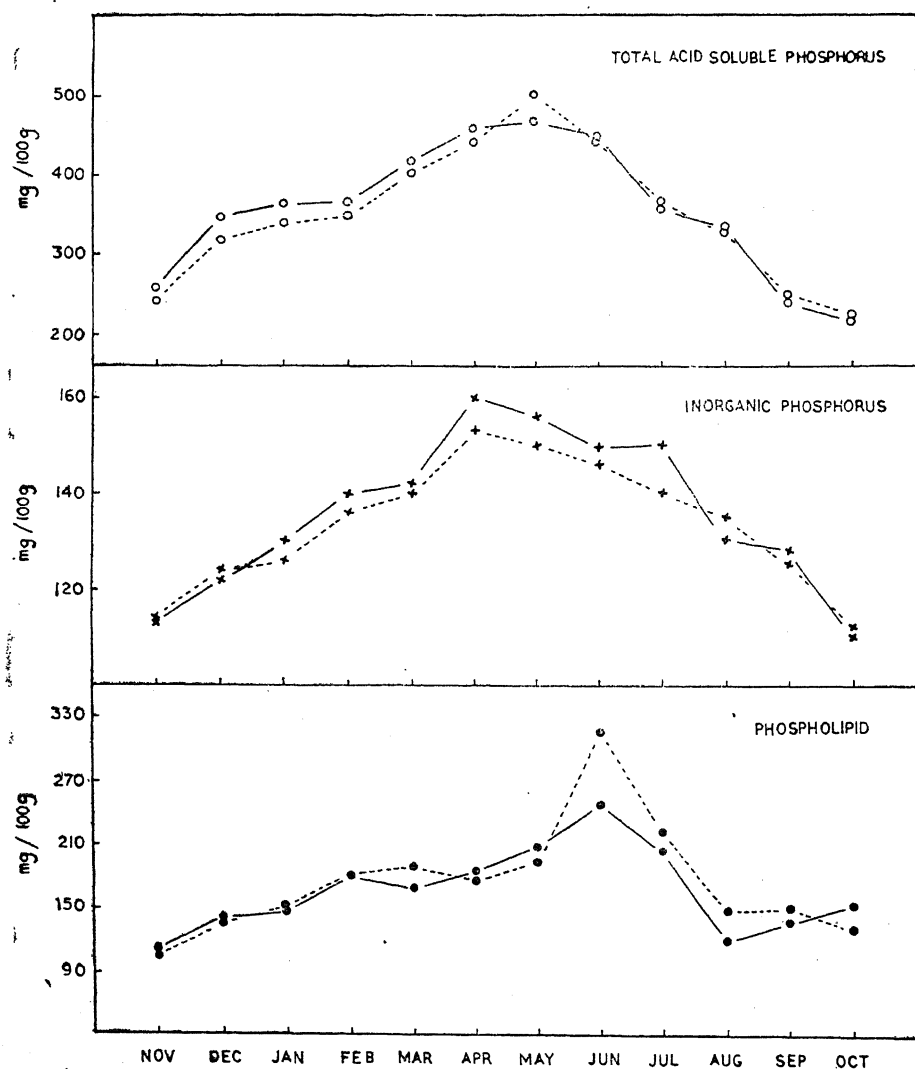


Figure 1. Seasonal variations in the phosphorus contents of the muscle of *Clarias batrachus* L.

evident from the figure, in both the sexes, the total acid soluble phosphorus values in the muscle were higher from April to June, the maximum being recorded in May. From June onwards there was a gradual decrease and the minimum values were recorded in October. Thereafter a regular rise was noted. Almost a similar trend of change was followed by inorganic phosphorus, being highest in April and lowest in October. The phospholipid content was highest in the month of June, the values in females were relatively higher than in males. A subsequent fall in phospholipid content occurred during July and August and the level remained low during winter period (September to January).

Mineral contents are influenced by a number of factors such as age, sex and sexual maturity (Vinogradov and Odum 1953). The present observed variations appear to be correlated mainly to feeding, gonad maturation and spawning. Though the synthesis of phosphorus contents takes place inside the body, their chief source outside the body is food. In *C. batrachus* higher values of total acid soluble phosphorus, inorganic phosphorus and phospholipid were recorded in the period (April, May and June) when feeding intensity of fish was high. This high rate of feeding indicates increased metabolic activity of fish during these months. Similar observations have been reported by other investigators (Siddiqui and Siddiqui 1965 ; Siddiqui 1972). Similarly low values observed during winter period appear to be the result of less active feeding.

There was a marked relationship between the muscle phosphorus contents and the cycle of gonad maturation. A gradual increase in total acid soluble phosphorus, inorganic phosphorus and phospholipid was recorded when gonads advanced towards maturity. The highest concentration in the muscle from April to June coincided with the period of peak ripeness. Thereafter the constant decline corresponded to the period of spawning and in spent fish, values were quite low (September, October). The fall in phospholipid content was from June to August. These findings are in accordance with the observation of Siddiqui and Siddiqui (1965) and Siddiqui and Naseem (1971). They reported maximum value of phosphorus content in the fish with ripe gonad and declining values in spawning fish.

It has been observed that during spawning period, feeding activity of fish is restricted and fish needs a great amount of energy. This energy is derived from various sources. Phosphorus content may be one of the sources as the inorganic and organic phosphorus play a very important role in energy transfer and enzyme system (Harper 1963). Hence a gradual depletion in different phosphorus contents during spawning is quite justifiable. Besides, through intermediary formation of lecithin, phosphorus is associated with fat metabolism and through the formation of hexosephosphates of adenylic acid and of creatine phosphate it plays a primary role in carbohydrate metabolism. During maturation cycle, variations have been reported in carbohydrate and fat contents (Valtonen 1975; Petersen and Emmersen 1977 ; Fernandez and Planas 1980).

Acknowledgement

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References

- Bano Y 1975 Variations in the chemical composition of different sections of the flesh of *Clarias batrachus* L.; *Indian J. Zool.* 3 39-42
- Bano Y 1977 Seasonal variations in the biochemical composition of *Clarias batrachus* L.; *Proc. Indian Acad. Sci.* 85 147-155
- Bano Y and Hameed T 1979 Seasonal changes in cholesterol content of the muscle of catfish *Clarias batrachus* L.; *Indian J. Exp. Biol.* 17 214-215
- Bhushana Rao K S P 1965 Biochemical studies on red and white muscles of *Caranx sexfasciatus* Quoy and Gaimard; *Proc. Indian Acad. Sci.* 62 87-91
- Chang V M and Idler D R 1960 Biochemical studies on sockeye salmon during spawning migration. XIII. The distribution of phosphorus compounds, creatine and inositol in the major tissues; *J. Fish. Res. Bd. Can.* 17 565-582
- Fernandez J and Planar J 1980 Annual variation of some carbohydrate and lipid parameters in the fish *Spicara chryselis* during captivity; *Comp. Biochem. Physiol.* 67 383-389
- Harper H A 1963 *Review of physiological chemistry* (Bombay: Lange Medical Publication)
- Jafri A K 1965 *Studies on the biochemical composition of some freshwater fishes*; Ph.D. Thesis, Aligarh Muslim University, Aligarh
- Nakano T 1960 Studies on the physiological chemistry of phosphorus compounds in fish muscle. II. On the individual and regional variations of phosphorus compound contents in fish muscle; *Bull. Jpn. Soc. Scient. Fish.* 26 1192-1197
- Nakano T and Tsuchiya Y 1960 Physiological chemistry of phosphorus compounds in fish muscle. I. Distribution of various phosphorus compounds in fish muscle; *Nippon Suisan Gakkaishi* 26 1095-1098
- Petersen I M and Emmersen B K 1977 Changes in serum glucose, lipids, liver glycogen and phosphorylase during vitellogenesis in nature in the flounder *Platichthys flesus* L.; *Comp. Biochem. Physiol.* B58 167-171
- Qayyum A and Qasim S Z 1964 Studies on the biology of some freshwater fishes. Part I. *Ophicephalus punctatus* Bloch; *J. Bombay Natl. Hist. Soc.* 61 74-98
- Shell E W 1961 Chemical composition of blood of small mouth bass; *U.S. Fish Wildlife Serv. Res. Rep. No.* 57 1-36
- Siddiqui A Q and Naseem S M 1971 Seasonal variations in the biochemical composition of blood serum of *Heteropneustes fossilis* (Bloch) (Teleostomi, Heteropneustidae); *Kashmir Science* 8 41-50
- Siddiqui M A and Siddiqui M A 1965 Seasonal variations in calcium, inorganic phosphate and alkaline phosphatase content of *O. punctatus* (Bloch); *Indian J. Exp. Biol.* 4 122-123
- Siddiqui N 1972 *Studies on the chemical constituents of the blood plasma of some freshwater fishes*; Ph.D. Thesis, Aligarh Muslim University, Aligarh
- Vinogradov A P and Odum V 1953 *The elementary chemical composition of marine organisms*; *Sears Foundation for Marine Research, Yale Univ., New Haven, Connecticut*
- Valtonen T 1974 Seasonal and sex bound variation in the carbohydrate metabolism of the liver of white fish; *Comp. Biochem. Physiol.* A47 713-727

The tannery industrial effluent effect on succinate dehydrogenase activity pattern in a freshwater snail, *Pila globosa*

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Abstract. A high degree of pollution by tannery effluent contamination has been recorded in an irrigation reservoir in North Arcot district, Tamil Nadu. Seepage of contaminants into drinking water wells has also been observed. The tannery effluent is found to inflict changes in succinate dehydrogenase activity levels in the hepatopancreas of *Pila globosa*, a common inhabitant of the polluted environment.

Keywords. *Pila globosa* ; tannery effluent ; chromium ; tannin ; succinate dehydrogenase.

1. Introduction

A major irrigation reservoir namely Chennasamudram reservoir of Chennasamudram village along with its hamlets and drinking water wells contaminated by tannery effluents were identified in Walajapet taluk, North Arcot District, Tamil Nadu. Physico-chemical analysis was carried out for a calendar year at the above work spot (Guruprasada Rao and Nanda Kumar 1981) which revealed that tannery effluents contain many toxic substances such as chromium compounds, tannins, sodium chloride, calcium chloride and other compounds in considerable quantities which adversely affect the biological systems thereby posing a threat to the ecosystem (Eye and Lawrence 1971). Hence an attempt is made in the present investigation to study the effect of untreated tannery effluent (TE) at different concentrations and also its toxic ingredients like chromium (VI), sodium chloride and other compounds on succinate dehydrogenase (SDH) activity pattern of a freshwater snail, *Pila globosa*, a common inhabitant of the polluted area which shows low resistance to polluted freshwater environment and is also found to be sensitive to chromium (Guruprasada Rao and Nanda Kumar 1982).

2. Materials and methods

Pila globosa were collected from uncontaminated water resources. They were acclimated to the laboratory conditions for eight days and maintained as reported

earlier (Muralimohan and Sasirababu 1976). The animals were exposed to the media reported earlier (Guruprasada Rao and Nanda Kumar 1982). The ratio of one animal in 500 ml of medium was maintained throughout the exposure period in a glass jar. The media employed consisted of different percentages of TE, and also media containing different concentrations of potassium chromate, sodium chloride, calcium chloride, and tannin (Wattle extract spray dried) prepared in freshwater as they form the main ingredients of TE. The animals were exposed to the above media at selected concentrations and for different periods separately as mentioned in table 1. After the exposure period, they were removed and the hepatopancreas were isolated on ice blocks and immediately transferred to the refrigerator maintaining an ambient temperature of 0° C. The tissue was used for assaying SDH activity. The concentration of the media chosen to expose the animals were either found in the effluent or in the reservoir water under natural conditions. These identifying concentrations chosen are indicated in table 1. Higher concentrations were also chosen to magnify the extent of implication on the enzyme chosen.

Hepatopancreas were homogenised in ice cold 0.25 M sucrose solution, centrifuged at 2,500 rpm and the supernatant used as enzyme source. Enzyme assay : SDH was assayed by the method of Nachlas *et al* (1960) while employing INT as electron acceptor and extracting formazan in toluene layers (Nanda Kumar *et al* 1973).

3. Results and discussion

The succinate dehydrogenase activity (SDH) showed an enhancement when the snails were exposed to TE for 3 and 24 hr. Whereas SDH activity showed a significant decrease when exposed for 10 days. Hence alteration in the enzyme activity level in animals exposed for short periods in the effluent contaminated environment cannot be taken as an index. The change in the enzyme activity level is an overall expression of combined action of all ingredients of TE. However a detailed study was done on the effect of various ingredients of tannery effluent separately on the SDH activity in snails. The effect of potassium chromate, tannin, sodium chloride and calcium chloride separately were studied on SDH activity. Potassium chromate at 3 hr enhanced the SDH activity (table 1) at various concentrations (1-100 ppm). The SDH activity level showed no increase at all concentrations chosen (table 1) at 3 hr period and also at 2.5 ppm level after 10 day period. However the decrease at 24 hr was not significant. Stimulation of dehydrogenase systems in rats (Horecker *et al* 1939), oxidation of NADH to generate NAD by potassium chromate (Gruber and Jennette 1978) and enhanced oxygen consumption (Ergeshev 1974 ; Sheer and Armitage 1973) with subsequent oxidation of citric acid metabolites may be cited for the observed increase in SDH activity.

Tannin was also found to enhance SDH activity (at 3 hr) with a subsequent depressing effect (24 hr and 10 days). Earlier reports of Luciani (1973) on SDH inhibition by tannic acid support the present investigation. Corroborative evidences of the inhibitory action of tannic acid on succinate transportation into rat liver mitochondria (Johnson 1972) in isolated erythrocytes (Mitzavilla *et al* 1977) also strengthen this observation.

Table 1. Succinate dehydrogenase activity levels in the hepatopancreas of *Pila globosa* exposed to different media (% change in enzyme activity is calculated from μ moles formazan formed/mg protein/hr).

Medium (1)	Concentration* (2)	Exposure time (3)	Per cent change in SDH activity level (4)
Tannery effluent	10%	3 hr	$+9.86 \pm 3.45$ N.S.
do.	10%	24 hr	$+17.69 \pm 1.52$ $P < 0.05$
do	10%	10 days	-16.43 ± 1.72 $P < 0.005$
Potassium chromate	1 ppm	3 hr	$+20.71 \pm 2.4$ $P < 0.05$
do.	10 ppm	3 hr	$+25.28 \pm 3.1$ $P < 0.05$
do.	50 ppm	3 hr	$+27.89 \pm 2.2$ $P < 0.001$
do.	100 ppm	3 hr	$+28.67 \pm 2.4$ $P < 0.05$
do.	25 ppm	24 hr	-15.98 ± 4.3 N.S.
do.	2.5 ppm	10 days	$+20.72 \pm 1.15$ $P < 0.05$
Tannin (Wattle extract spray dried)	100 ppm	3 hr	$+30.76 \pm 3.0$ $P < 0.05$
Tannin	100 ppm	24 hr	-5.30 ± 0.48 $P < 0.05$
do.	20 ppm	10 days	-19.76 ± 2.34 $P < 0.05$
Sodium chloride	5000 ppm	3 hr	$+9.42 \pm 4.2$ N.S.
do.	5000 ppm	24 hr	$+8.76 \pm 1.17$ $P < 0.05$
do.	1000 ppm	10 days	-31.16 ± 3.67 $P < 0.05$
Calcium chloride	1000 ppm	3 hr	$+2.55 \pm 1.5$ N.S.
do.	1000 ppm	24 hr	$+1.28 \pm 1.1$ N.S.
do.	500 ppm	10 days	$+13.99 \pm 1.61$ $P < 0.05$

+ or — indicate % increase or decrease in enzyme activity over control respectively.
Control activity is normalised to $100\% = 0.0527 \pm 0.001 \mu$ moles of Formazan formed/mg protein/hr.

\pm S.D. from mean of six observations.

N.S. Not significant at the level of 5%.

* Concentrations comparable to effluent/irrigation reservoir water (Guruprasada Rao and Nanda Kumar 1981).

Sodium chloride enhanced the SDH activity at 3 and 24 hr whereas at 10 day the SDH activity showed a decrease. Variations in the salinity of the environmental medium are known to exert considerable influence on the activity behaviour and metabolism of invertebrates (Gilles *et al* 1971; Negus 1968). Sodium chloride was found inhibitory to TCA cycle (Korff *et al* 1954) and to SDH *in vitro* (Gilles *et al* 1971). The decrease in the SDH activity in *Pila globosa* at 10 day exposure observed in the present investigation might be due to the accumulation of chloride ion in the medium. Corroborative evidence comes from the works of Venkata Reddy (1976) who demonstrated a decrease in hepatopancreas SDH activity in crab under sodium chloride stress.

Enhancement in the SDH activity was observed in *Pila globosa* exposed to calcium chloride (table 1). At present experimental evidence is lacking on the calcium chloride stimulation of SDH system. However it is suggested that the triggering of glycolytic pathway, activation of ATP hydrolysis and increase in glucose amounts (Hochachka and Somero 1973; Meenakshi 1956) might be responsible for generation of raw material required for oxidative metabolism (Adams and Quastel 1956; Fruton and Simmonds 1965) and the possible increase in SDH system.

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References

- Adams D H and Quastel J H 1956 *Proc. Roy. Soc.* **B145** 472-479
- Ergeshev I 1974 Chromium effect on the intensity of O₂ consumption by guinea pigs; *Biol. Abstr.* **58** 28980
- Eye J D and Lawrence L 1971 Treatment of wastes from a sole leather tannery; *J. Wat. Pollut. Cont. Fed.* **43** 2291-2303
- Fruton J S and Simmonds S 1965 *General biochemistry* (Madras: Asia Publishing House)
- Gilles R, Hogue P and Kearny B 1971 Effects of various ions in the succinate dehydrogenase activity of *Mytilus californianus*; *Life Sciences* **10** 1421-1427
- Gruber J E and Jennette K W 1978 Metabolism of carcinogen chromate by rat liver microsomes; *Biochem. Biophys. Res. Commun.* **82** 700-706
- Guruprasada Rao M and Nanda Kumar N V 1981 Analysis of irrigation reservoir contaminated by tannery effluents; *Indian J. Environ. Health* **23** 239-241
- Guruprasada Rao M and Nanda Kumar N V 1982 The tannery industrial effluent effect on cholinesterase activity pattern in a freshwater snail, *Pila globosa*; *J. Environ. Biol.* (in press)
- Hochachka P W and Somero G N 1973 *Strategies of biochemical adaptation* (Philadelphia: W B Saunders Company) pp. 132-143
- Horecker B L, Stotz B and Hogness T R 1939 The promoting effect of chromium and the rare earths in the succinic dehydrogenase cytochrome system; *J. Biol. Chem.* **128** 251-256
- Johnson D J 1972 Effects of tannic acid on ion transport in rat liver mitochondria; *Arch. Biochem. Biophys.* **151** 316-321
- Korff R W V, Macpherson E H and Glaman G V 1954 Potassium ion stabilization of respiration by heart muscle mitochondria; *J. Biol. Chem.* **209** 151-153

- Luciani S 1973 Inhibition by tannic acid of succinate and malate translocation across mitochondrial membrane ; *Biochem. Pharmacol.* **22** 1821-1828
- Meenakshi V R 1956 Studies on physiology of *Pila virens* (Lamarck) with special reference to aestivation. Ph.D. Dissertation, Annamalai University
- Mitzavilla S, Blanquat G D and Derache R 1970 Effect of tannic acid on intestinal absorption in the mouse ; *Food Cosmet. Toxicol.* **8** 27-33
- Muralimohan P and Sasirababu K 1976 Modification of enzyme activities by body fluids, amino acids and divalent cations in the nervous system of aestivating snail, *Pila globosa*; *Indian J. Exp. Biol.* **14** 232-238
- Nachlas M. M 1960 A colorimetric method for the determination of succinic dehydrogenase activity ; *J. Biol. Chem.* **235** 499-503
- Nanda Kumar N V, Radhakrishnamurthy Ch, Vijayakumari D and Swami K S 1973 Axonal protein changes and succinate dehydrogenase activity in sheep medulla oblongata ; *Indian J. Exp. Biol.* **11** 525-528
- Negus M R S 1968 Oxygen consumption and amino acid levels in *Hydrobis ulvae* (Pennat) in relation to salinity and behaviour ; *Comp. Biochem. Physiol.* **24** 317-325
- Sheer C A and Armitage K B 1973 Preliminary studies of the effects of dichromate ion on survival and O₂ consumption of *Daphnia pulex* ; *Crustaceana* **25** 51-69
- Venkata Reddy V 1976 Studies on mechanisms underlying acclimation to salinity in the fresh-water field crab, *Paratelphusa hydrodromous* ; Ph.D. Thesis (Tirupati : SV University)

Durational effects of hemispaying on ovarian hypertrophy and estrous cycle in albino rats

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Abstract. Ovarian hypertrophy is studied by hemispaying the rats for 7, 15, 20, 25 and 30 days. The compensatory hypertrophy of the ovary is calculated in relation to their respective sham operated controls. The maximum hypertrophy is observed 20 days after hemispaying, as indicated by ovarian weight and its histological observations. Thereafter the hypertrophic response though significant, decreases gradually, indicating that once the circulating estrogen secreted by the hypertrophied ovary comes to preoperative level, the pituitary gonadotrophin level also falls down. The hemispaying has no significant effect either on the duration or number of estrous cycle.

Keywords. Ovarian compensatory hypertrophy; hemispaying; estrous cycle.

1. Introduction

Unilateral ovariectomy or hemispaying causes compensatory follicular proliferation, ovulation and hypertrophy in rats, mice and hamsters (Arai 1920; Mandl and Zuckerman 1951; Greenwald 1961; Pepler 1975). This may be due to the unchanged availability of pituitary gonadotrophins (FSH and LH) to the remaining single ovary and/or increase in the pituitary release of gonadotrophins due to decrease in the circulating estrogen after semiovariectomy (Edgren *et al* 1965; Welchen 1970, 1972; Howland *et al* 1974). The compensatory response of the ovary may continue till the ovary gets the increased amount of pituitary gonadotrophins and once the gonadotrophins level falls down to normal, due to increase in the steroid output by the hypertrophied ovary, this compensatory hypertrophy may also decrease. Therefore, the present investigation is to study the duration required to obtain the optimum hypertrophy in albino rats.

2. Material and methods

Nulliparous, female albino rats of Holtzman strain, with regular established estrous cycle, weighing 130-150 g, 70-80 days old were hemispayed. The right

ovary was exposed by dorsolateral route, major blood vessels were ligated and after split opening the bursa, the ovary was carefully removed. Sham operation was performed by just exposing the right ovary. All operations were carried at estrous, under mild ether anaesthesia.

The experimental rats were maintained in individual cages, with Hindustan Lever rat feed, at water *ad libitum*, at a room temperature of $27 \pm 1^\circ \text{C}$ and 12 hr light/darkness.

The estrous cycle of all the experimental rats were studied everyday morning by vaginal smear observations. The rats were autopsied after 7, 15, 20, 25 and 30 days. Ovaries were dissected out free from adherent tissue, weighed, fixed in Bouin's fluid, sectioned and stained with heamatoxylin eosin.

3. Results

3.1. Ovarian hypertrophy

The present investigation is to study the durational effects of hemispaying on ovarian hypertrophy. In sham operated controls, there is no appreciable change in the ovarian weight from day 7 to 30. In hemispayed rats compensatory hypertrophy is observable as early as 7 days after the operation, wherein per cent hypertrophy is 25.56 ($P < 0.1$). This hypertrophic response gradually increases by 15 and 20 days wherein the respective per cent hypertrophy is 50.41 ($P < 0.01$) and 96.40 ($P < 0.001$) in relation to respective sham operated controls. Thereafter though the ovarian hypertrophy is significant as evidenced by the hemispaying for 25 and 30 days wherein 65.54% ($P < 0.001$) and 64.74% ($P < 0.001$) hypertrophy, is seen respectively, it is slightly less compared to that of 20 days (table 1).

Table 1. Durational effect of hemispaying on ovarian hypertrophy in albino rats.

Duration (days)	Ovary wt. mg/100 g		% hypertrophy
	Sham operated	Body wt. M \pm S.E. Hemispayed	
7	17.18 \pm 0.95	21.57 \pm 1.91*	25.56
15	18.46 \pm 1.85	27.77 \pm 0.98**	50.43
20	15.40 \pm 0.30	30.24 \pm 1.94***	96.40
25	16.92 \pm 0.94	28.06 \pm 1.99***	65.54
30	17.73 \pm 0.26	29.31 \pm 1.14***	64.74

% hypertrophy is calculated in relation to respective sham operated controls.

M \pm S.E. = Mean \pm standard error.

* $P = 0.1$; ** $P = 0.01$; *** $P = 0.001$.

3.2. Ovarian weight

The ovarian weight in the hemispayed rats also goes on increasing from day 7 to 20, and by day 20 the ovarian weight is almost doubled in hemispayed rats (30.24 ± 1.94 mg) in relation to their sham operated controls (15.40 ± 0.30 mg) with 96.40% compensatory hypertrophy. Then onwards gradually the ovarian weight falls down along with a decrease in the ovarian compensatory hypertrophy.

3.3. Ovarian histology

Histological observations indicate that the initiation of the ovarian hypertrophy after hemispaying begins as early as 7 days, wherein the ovary shows large corpora lutea and graafian follicles. Significant ovarian hypertrophy is seen by 15 days, but it is maximum by 20 days wherein the ovaries are large with well developed corpora lutea and graafian follicles, indicating the increased follicular proliferation and ovulation. Similar observations in the ovarian histology is made after 25 days and 30 days of hemispaying, though the ovarian hypertrophic response is slightly reduced.

3.4. Estrous cycle

The cyclical changes observed in the study of estrous cycle gives a fair index of the ovarian activities (table 2). In the present experiment hemispaying has no significant effect on estrous cycle either in the duration of diestrus or on the number of estrous cycles. In sham operated rats the duration of diestrus ranges from 2.8 to 3.0 days whereas it is 2.5 to 3.1 days in hemispayed rats. The number of estrous cycles goes on increasing gradually with the increase in the

Table 2. Durational effect of hemispaying on estrous cycle in rats.

Duration (days)	duration of diestrus M \pm S.E.		Number of cycles M \pm S.E.	
	Sham operated	Hemispayed	Sham operated	Hemispayed
7 (5)	3.0 \pm 0.0	3.1 \pm 0.2	1.0 \pm 0.0	1.2 \pm 0.2
15 (5)	2.9 \pm 0.5	2.0 \pm 0.3	2.8 \pm 0.2	2.8 \pm 0.2
20 (5)	2.8 \pm 0.3	2.5 \pm 0.1	3.0 \pm 0.2	4.0 \pm 0.0
25 (5)	3.0 \pm 0.2	3.1 \pm 0.3	4.4 \pm 0.2	4.6 \pm 0.1
30 (5)	2.7 \pm 0.2	2.9 \pm 0.2	6.0 \pm 0.3	5.8 \pm 0.2

M \pm S.E. = Mean \pm standard error.

Number in parenthesis denotes the number of rats,

duration of the experiment in both sham operated and hemispayed rats. The number of estrous cycles ranges from 1.0 to 6.0 from day 7 to 30. It is evident from the above results that these rats are regular 5 days cyclers with 3 days of diestrus. The hemispayed rats though having single ovary can maintain the hormonal balance which is essential for the vaginal cornification.

4. Discussion

Ovarian compensatory hypertrophy and ovulation after hemispaying is observed by several investigators in rats, mice, hamsters and guinea pigs (Arai 1920 ; Greenwald 1961 ; Hermerck and Greenwald 1964 ; Peppler 1975). The compensatory hypertrophy is evident even in the neonatal rats, pregnant and pseudo-pregnant rats, but not so apparent in aged rats, since there is a decline in the pituitary output of FSH and LH during that period (Labhsetwar 1967, 1969 ; Chatterjee and Greenwald 1971; Peppler 1971). In spite of several investigations the mechanism of ovarian compensatory hypertrophy is still debatable. It is alluded to relative increase in the availability of serum gonadotrophins to the remaining single ovary after hemispaying, since no increase in the pituitary gonadotrophins is observable after hemispaying (McLaren 1963, 1966 ; Edgren *et al* 1965). However, this contention is questioned as there is an increase in the gonadotrophin output, due to decrease in the circulating estrogen after hemispaying which is responsible for the ovarian compensatory hypertrophy (Grady and Greenwald 1968 ; Benson *et al* 1969 ; Walshen 1970, 1972 ; Howland and Skinner 1973). But according to Greenwald (1968) and Peppler (1972) the mechanism of ovarian hypertrophy involves not only an increase in the output of pituitary gonadotrophins, but also the time of exposure to the available gonadotrophins.

In this paper the ovarian compensatory hypertrophy in relation to sham operated controls is enhanced with the duration of hemispaying. Therefore the ovarian compensatory hypertrophy obtained after 7 days is not significant ($P < 0.1$), significant after 15 days ($P < 0.01$) and highly significant thereafter ($P < 0.001$). These results agree with those of Greenwald (1968) and Peppler (1972), wherein the significant compensatory hypertrophy is obtained with an increase in the time of exposure of the ovary to the constant gonadotrophic levels. The maximum ovarian hypertrophy is observed by 20 days after hemispaying. These results appear to be in agreement with those of Benson *et al* (1969), wherein an increase in the initial surge of serum FSH is seen on day 4, comes to preoperative levels by day 20 to 24. Therefore ovarian hypertrophy increases up to day 20 and once the circulating estrogens come to preoperative level there will be no increase in the gonadotrophin output, hence the hypertrophic response of the ovary also decreases after 20 days.

The study of estrous cycle indirectly indicates the gonadotrophins output from the pituitary, preceded by the ovarian estrogen secretion. In the present investigation hemispaying has no effect on the duration of estrous cycle wherein the diestrus extends from 3-4 days both in sham operated and hemispayed rats, which is in full agreement with the opinion held by Greenwald (1960) and Peppler and Greenwald (1970). The number of estrous cycles increases from 1 to 6 as the

duration of the experiment increases from day 7 to 30, in both sham operated and hemispayed rats. This indicates that the steroid hormone production from the remaining single ovary in hemispayed rats is sufficient for the vaginal cornification even before the significant ovarian hypertrophy takes place.

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References

- Arai A 1920 On the cause of the hypertrophy of the surviving ovary after hemispaying (albino rats) and on the number of ova in it; *Am. J. Anat.* **28** 59-79
- Benson B, Sorrentono B and Evans J S 1969 Increase in serum FSH following unilateral ovariectomy in rat; *Endocrinology* **84** 369-374
- Chatterjee A and Greenwald G S 1971 Compensatory ovarian hypertrophy of the pseudo-pregnant and pregnant rats; *Endocrinology* **88** 191-196
- Edgren R A, Parlow A F, Peterson D L and Jones R C 1965 On the mechanism of ovarian hypertrophy following hemicastration in rats; *Endocrinology* **76** 97-102
- Grady K L and Greenwald G S 1968 Studies on the interaction between the ovary and pituitary follicle stimulating hormone in golden hamsters; *J. Endocrinol.* **40** 85-90
- Greenwald G S 1960 The effect of unilateral ovariectomy on follicular maturation in the hamster; *Endocrinology* **66** 89-95
- Greenwald G S 1961 Quantitative study of follicular development in the ovary of intact and unilaterally ovariectomised hamsters; *J. Reprod. Fertil.* **2** 351-361
- Greenwald G S 1968 Influence of one or two ovaries on ovulation and ovarian weight in the hypophysectomised rat; *Endocrinology* **82** 591-596
- Hermerck A S and Greenwald G S 1964 The effect of unilateral ovariectomy on follicular maturation in guinea pigs; *Anat. Rec.* **148** 171-176
- Howland B E and Skinner K R 1973 Effect of hemiovariectomy on serum FSH and LH levels during the estrous cycle in the rat; *J. Reprod. Fertil.* **32** 501-503
- Howland B E, Jack M I and Beaton D B 1974 Effect of hemiovariectomy and strain of rat on serum gonadotrophin levels; *Experientia* **30** 653-654
- Labhsetwar A P 1967 Age dependent changes in the pituitary-gonadal relationship: A study of ovarian compensatory hypertrophy; *J. Endocrinol.* **39** 387-393
- Labhsetwar A P 1969 Age dependent changes in the pituitary-gonadal relationship II: A study of pituitary FSH and LH content in the female rat; *J. Reprod. Fertil.* **20** 21-28
- Mandl A M and Zuckerman S 1951 Number of normal and atretic oocytes in unilaterally spayed rats; *J. Endocrinol.* **6** 112-119
- McLaren A 1963 Mechanism of ovarian compensation following unilateral ovariectomy in mice; *J. Reprod. Fertil.* **6** 321-322
- McLaren A 1966 Regulation of ovulation rate after removal of one ovary in mice; *Proc. R. Soc. B* **166** 316-340
- Peppler R D 1971 Effect of unilateral ovariectomy on follicular development and ovulation in cycling, aged rats; *Am. J. Anat.* **132** 423-429

- Peppler R D 1972 FSH and LH levels in the intact and unilaterally ovariectomized cycling rat; *Acta Endocrinol. (kbh)* **69** 267-280
- Peppler R D 1975 Effect of removing one ovary and a half on ovulation number in cycling rats; *Experientia* **31** 243-245
- Peppler R D and Greenwald G S 1970 Effects of unilateral ovariectomy on ovulation and cycle length in 4- and 5-day cycling rats; *Am. J. Anat.* **127** 1-8
- Welchen R 1970 Compensatory ovarian growth and compensatory ovulation after unilateral ovariectomy in rats with an ovarian autograph in the region of the portal vein; *Acta Endocrinol. (kbh)* **65** 509-516
- Welchen R 1972 Effect of unilateral ovariectomy on follicular growth in hypophysectomized rats treated with pregnant mare serum gonadotrophins (37518); *J. Endocrinol.* **55** 227-228

Structure and seasonal changes in the testes of a freshwater crab, *Potamon koolooense* (Rathbun)

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Abstract. The paired 'H'-shaped testes of *Potamon koolooense* show histomorphological changes during various stages of maturity. Seminiferous tubules show different stages of spermatogenesis. A few undifferentiated or resting spermatogonia supply a new crop of germ cells for the next breeding season. In a tubule the meiotic divisions occur more or less synchronously in all the primary or secondary spermatocytes. Sperm consists of a head and mitochondrial vesicle which encloses axial filament and distal centrosome. Spermatogenetic activity is seasonal. Spermatogenesis begins in January-February, progresses slowly through March, reaching its peak in April-May. However, all the tubules do not mature simultaneously. Spermiation occurs during May and June, the spermatogenesis ceases gradually, and by December the testes enter a brief period of rest.

Keywords. *Potamon koolooense* ; testes ; histology ; seasonal changes.

1. Introduction

The structure of male reproductive organs in crustacea has been described by Spalding (1942), Cronin (1947), King (1948), Ryan (1967), Wolfe (1971), Chiba and Honma (1971) and Gupta and Chatterji (1976). There has been little information about the seasonal histomorphological changes in the testes of crustaceans as investigators concerned themselves with specific study, such as spermatogenesis (Binford 1913 ; Fasten 1926 ; Baker and Rosof 1927 ; Nath 1932) or measurement of male gonad index for assessment of reproductive cycle (Subrahmanyam 1963 ; Rahman 1967 ; Chandran 1968). Recently seasonal histological changes have been reported in the crab, *Pachygrapsus crassipes* (Chiba and Honma 1972), *Barytelphusa cunicularis* (Diwan and Nagabhushanam 1974) and crayfish, *Orconectes limosus* (Wielgus 1976). The present paper describes the seasonality in the testicular activity in a freshwater crab, *Potamon koolooense*.

2. Material and methods

15 to 20 live specimens of adult male *P. koolooense* (carapace width 37 mm to 45 mm) were collected every month during 1976-78, from a stream near

Pithoragarh. The weight of each specimen was recorded immediately before dissection. The testes were removed and placed immediately in fixative. Their length and weight were recorded after fixation. The gonad index (GI) was calculated using the formula (Giese 1959) :

$$GI = \frac{\text{weight of the gonad}}{\text{weight of the animal}} \times 100.$$

For histology, different regions of the testes were fixed in Bouin's fluid, Allen's Bouin solution or Helly's fluid. Paraffin sections of 5–6 μm thickness were cut and stained with Delafield's haematoxylin or Mayer's haemalum, using eosin as counterstain in all the cases. Heidenhain's Azan, Mallory's triple stain and periodic acid Schiff (PAS) were also used.

3. Observations

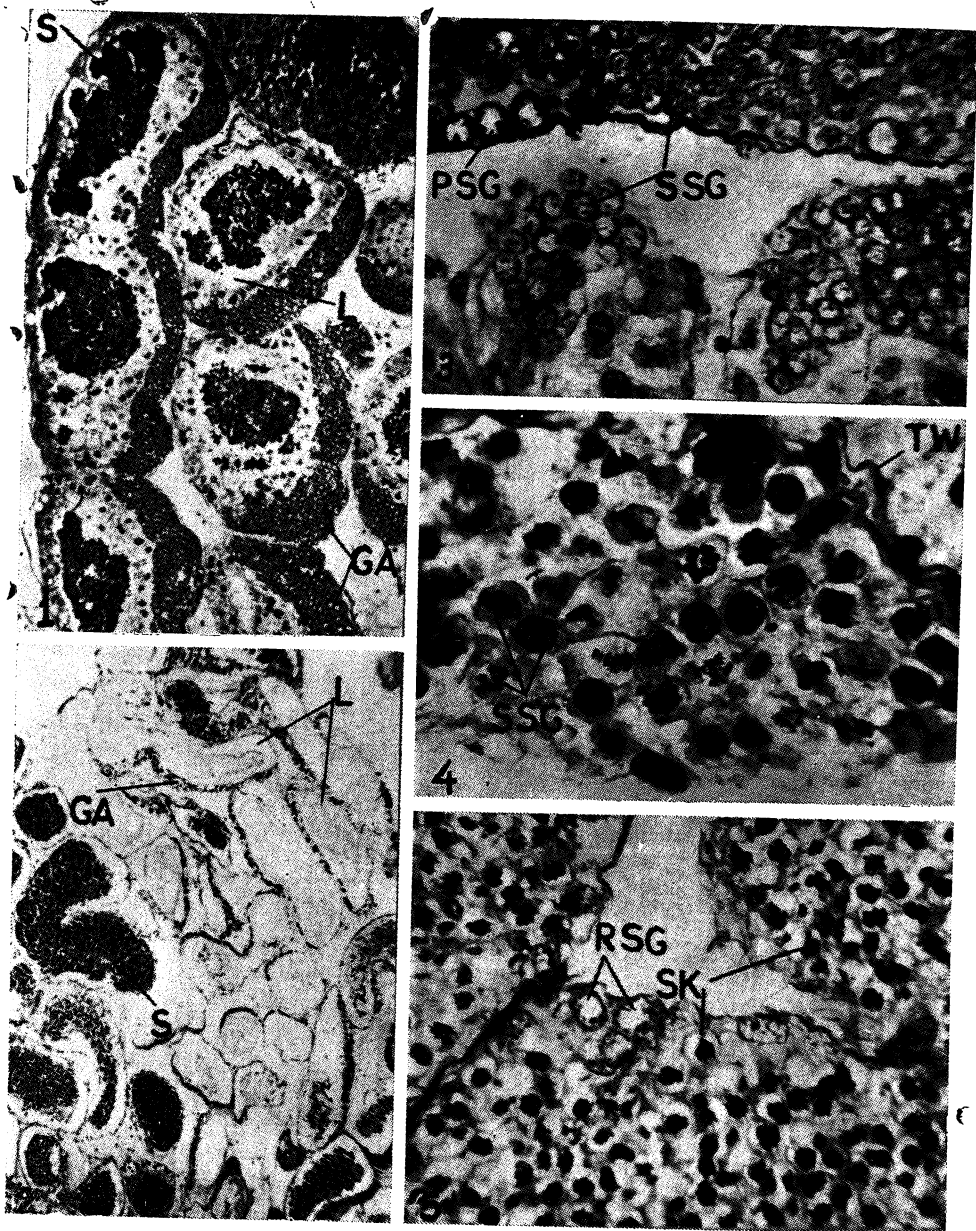
3.1. Morphology

The testes are paired elongated bodies, lying attached with the hypodermis of the overlying carapace but the ventral side freely rests upon the hepatopancreas. Testes of both the sides are connected together at their middle region by a cross band of testicular tissue so that the pair appears 'H'-shaped. Occasionally the testes of two sides are unequal in length and thickness. Each testis leads into a long highly coiled vas deferens which opens outside through the penis situated on the ventral subterminal region of the coxal segment of 5th leg.

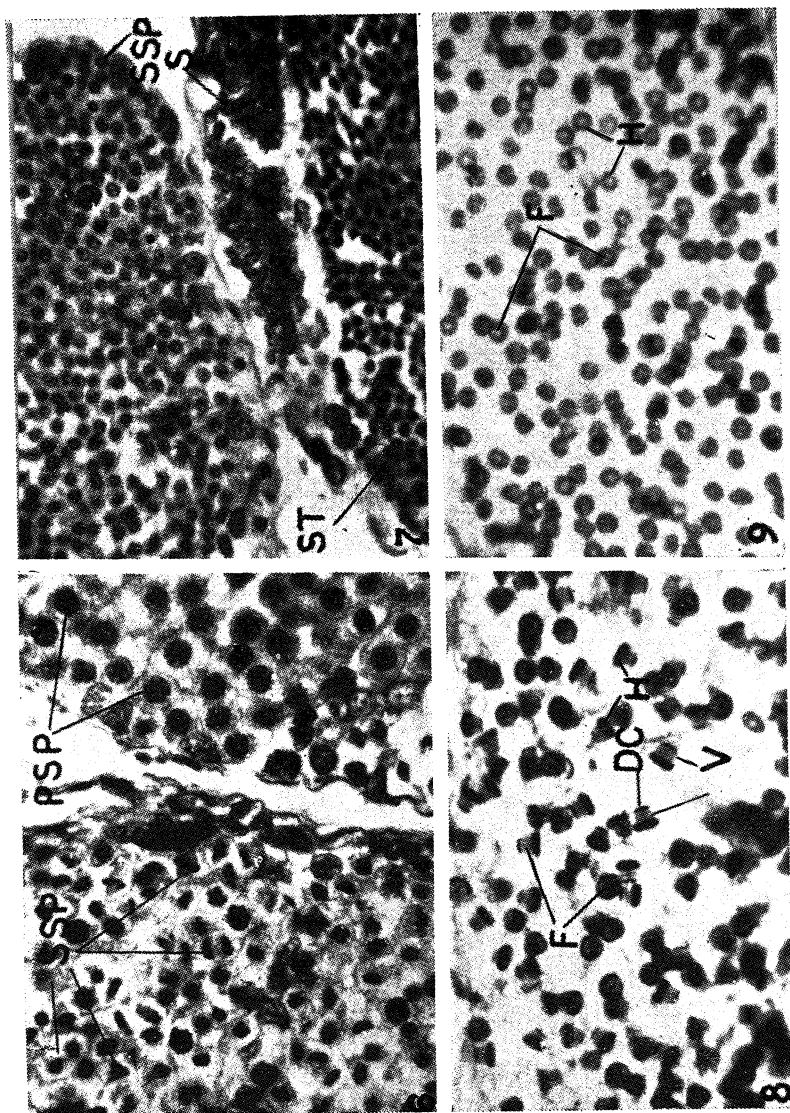
3.2. Histology

Each testis is made up of numerous convoluted seminiferous tubules of varying sizes, held together by a thin layer of connective tissue. The intertubular space contains a few blood vessels. Each tubule is covered by a thin layer of connective tissue, and in a transverse section shows two distinct areas, the germinative region and the lumen (figure 1). Different tubules in the same section consist of germ cells in different stages of development (figures 6, 7). Tubules at posterior region of testes become narrow and have small or no germinal area, while their lumen is full of sperms (figure 2). The tubules appear to be continuous, opening directly into the vas deferens.

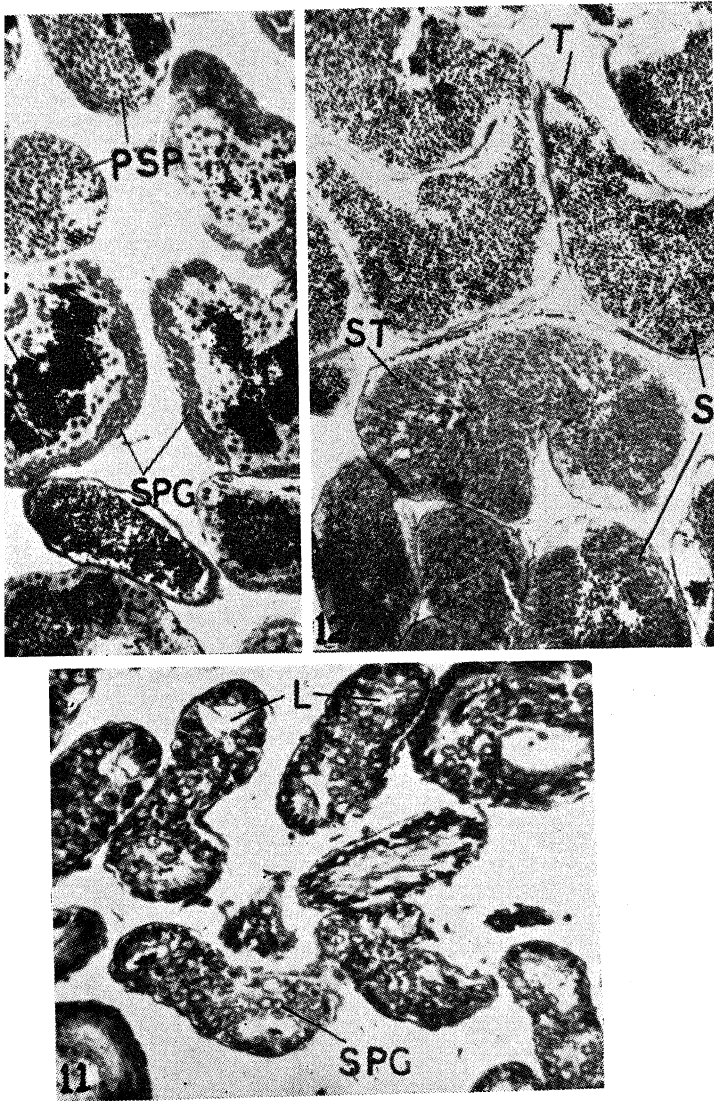
In spermatogenesis, the sperm mother cells or primary spermatogonia are the germ cells of first stage and are the largest of all. Each spermatogonium contains a thin rim of cytoplasm around a vesicular nucleus containing peripheral chromatin granules (figure 3). The number of primary spermatogonia gradually increases soon after spawning (June), becoming abundant during November and December (figure 11). Most of them later on divide mitotically and give rise to the secondary spermatogonia that will differentiate into spermatocytes, but a few remain undifferentiated till sperm formation and spermiation (figure 5). These are the resting spermatogonia which divide soon after spermiation and supply a new crop of germ cells for the next breeding season. The secondary spermatogonia are smaller than the primary ones and chromatin granules distributed homogeneously in nucleoplasm (figure 3). These cells undergo mitotic division (figure 4), so that a large number of primary spermatocytes are formed.



Figures 1-5. Portions of sections of testes. 1. Showing seminiferous tubules (T) in May (stage IV) $\times 90$. 2. Showing tubules of posterior testis, the germinal area (GA) is reduced $\times 40$. 3. Tubules showing primary (PSG) and secondary spermatogonia (SSG), July specimen $\times 380$. 4. Showing dividing spermatogonia (SSG) stage $\times 380$. 5. Showing primary spermatocytes at syneysis knot (SK) stage $\times 380$. (GA, Germinal zone; L, Lumen; RSG, Residual spermatogonia; S, Sperms, TW, Tubular wall.)



Figures 6-9. Portions of sections of testes. 6. Showing primary (PSP) and secondary spermatocytes (SSP) bearing tubules $\times 380$. 7. Showing secondary spermatocytes (SSP), spermatids (ST) and sperms (S) formed in April $\times 380$. 8. Showing structure of sperms $\times 610$. 9. Top view of sperms, stained with PAS $\times 610$. (PC, Distal centrosome; F, Axial filament; H, Head; V, Mitochondrial vesicle.)



Figures 10-12. Portions of sections of testes. 10. Showing stage I in July $\times 90$.
Showing tubules filled with spermatogonia in December (stage I) $\times 140$.
Showing tubules packed with spermatids (ST) and sperms (S) in May (stage
II) $\times 90$. (L, Lumen; PSP, Primary spermatocytes; RS, Residual sperms; SPG,
spermatogonia; T, Tubules.)

The primary spermatocytes are smaller than the spermatogonia but have enough eosinophilic cytoplasm and basophilic chromatin threads in the nucleoplasm (figure 6). A secondary spermatocyte is nearly half the size of the primary spermatocyte and its cytoplasm is poorly stainable but cell boundary is clearly visible in newly formed cells. The nuclei are small and have dense chromatin. This stage is of short duration, as cells of all stages from prophase to cytokinesis are found in the same tubule (figure 6). It is interesting to note that meiotic division occurs synchronously in all the primary spermatocytes of a tubule and the same is true for secondary spermatocytes. This is indicated by the presence of all the primary or secondary spermatocytes of a tubule at approximately the same stage of development (figures 5, 6). Furthermore, a tubule at any time contains either one type of spermatocytes or spermatids (figures 5, 6, 7).

The spermatids are small rounded bodies having deeply stained nuclei (figure 7), the cytoplasm stains grey with haematoxylin eosin. During spermiogenesis they undergo morphological changes and at the end dome-shaped sperms are formed. Each sperm consists of a head and the so-called mitochondrial vesicle (figure 8). The head is deeply stainable with haematoxylin and represents the nucleus. The vesicle is eosinophilic and contains a feebly staining axial filament. At distal tip of axial filament is a thick blue-black staining transverse piece, the so-called distal centrosome (figure 8). The proximal centrosome is not visible as it is reported to be fused with nucleus. Pseudopodial rays which spread out from head were not visible with the methods employed. When stained with PAS all the cells except spermatids and sperms are negative to the stain. In sperms both head and vesicle are PAS positive, while the axial filament is PAS negative (figure 9).

3.3. *Seasonal cycle*

The annual testicular cycle of *P. kooloense* can be divided into the following four stages on the basis of histomorphological characters :

3.3a. *Stage I (July to December)* : The size and weight of testes gradually decreases reaching a minimum value in November and December (figure 13). The testes are thin and translucent during September to December. On puncturing a milky seminal fluid comes out through the vas deferens. Some of the tubules have large number of spermatogonia and few residual sperms (figures 3, 10). Spermatogenesis still continues to exist in some of the tubules of the same section (figure 10), but the number of spermatocytes and spermatids decreases gradually and finally absent during December. This indicates gradual cessation of spermatogenesis in testes. The spermatogonia greatly increased in most of the tubules in October and were in preponderance during November and December (figure 11). During this period the dimension of tubules decrease and the wall of tubules becomes thick and undulated (figure 11).

3.3b. *Stage II (January to March)* : The spermatogenesis begins during January and February with an accompanying increase in size, weight and opaqueness of testes. Tubules slightly increase in diameter and their walls become comparatively thin. They contain a few primary spermatogonia and a large number of secondary spermatogonia. Mitotic figures are generally seen in such tubules

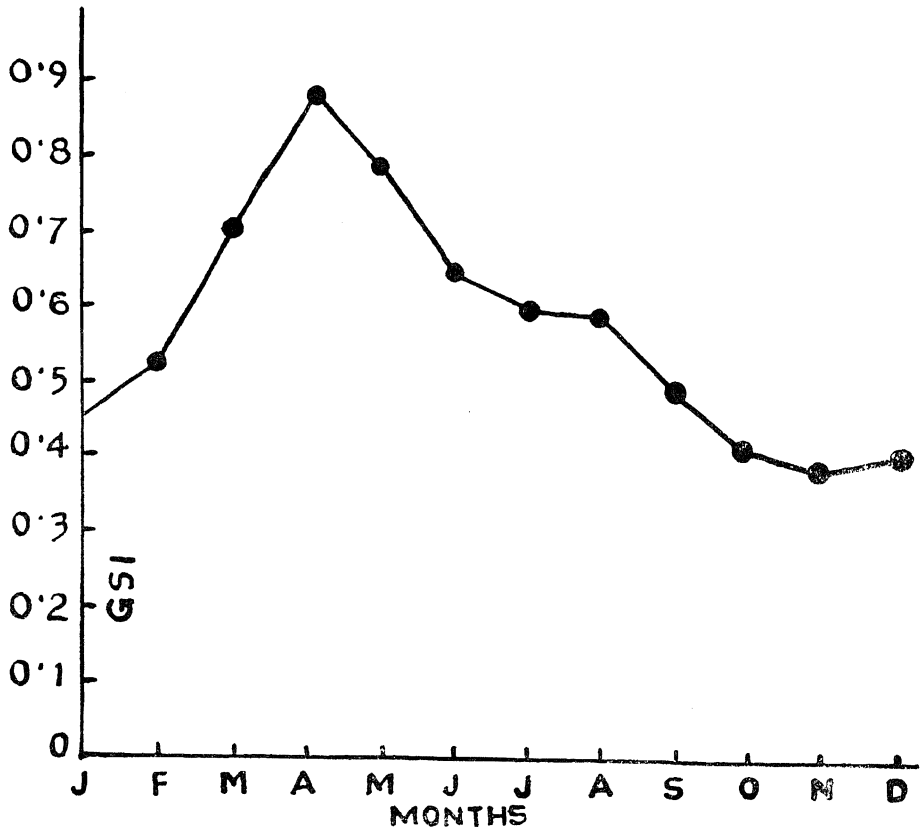


Figure 13. Showing seasonal changes in the gonad index (GSI) of male *P. koolooense*.

(figure 4). The primary and secondary spermatocytes are produced in some of the tubules. A few residual sperms are still retained in the lumen of the tubules and almost disappear in March. During March the spermatogonial population decreases and actively dividing primary and secondary spermatocytes become dominating cells in the tubules (figure 6). In a few tubules spermatids are formed but sperms are not yet developed.

3.3c. *Stage III (April to May)*: The testes have greatly increased in their size and weight, being maximum during April (figure 13). They appear turgid and opaque and their wall becomes so thin that the seminiferous tubules are visible. The vas deferens also appears swollen, opaque and highly coiled and when ruptured the seminal fluid does not ooze out from it. This stage is characterized by spermatogenetic and spermiogenetic activity. Spermatids and sperms are in preponderance (figure 7). Sperms are developed for the first time in April and tubules fully packed with sperms are seen in April and May (figure 12). Owing to this the tubules are greatly enlarged and turgid and as a result their walls become thin and intertubular spaces are decreased. However, the maturational

changes do not occur simultaneously in all the tubules of testes, as most of the tubules are filled with spermatids and sperms while some others are still at primary or secondary spermatocyte level. This results in the production of sperms in successive waves.

3.3d. *Stage IV (May to June)* : During this stage, the testes appear opaque and vas deferens is packed with seminal fluid. Both maturing and mature tubules are seen in the same section. Maturing tubules consist of dividing primary or secondary spermatocytes or spermatids and produce sperms a little later. Mature tubules undergo spermiation in May or June. In some of the specimens collected during May, testes show decrease in size and weight (figure 13). Some of the tubules contain primary spermatogonia and residual sperms (figure 1). This indicates that spermiation has taken place. All the specimens collected during June show spawned conditions in a number of tubules of their testes.

Gonad index is minimum in November–December and reaches a peak in April and is in conformity with the histomorphological changes in the testes (figure 13).

4. Discussion

The above study on the testes of *P. koolooense* reveals many interesting features. In the testes of crustaceans studied so far, the spermatogenetic cells are confined in discrete bodies which are variously described as cysts, clusters, seminiferous tubules or lobules, each containing germ cells at different stages of spermatogenesis (Binford 1913 ; Fasten 1926 ; Baker and Rosof 1927 ; Ryan 1967 ; Gupta and Chatterji 1976) or at the same stage of maturation (Iyer 1933 ; Wolfe 1971 ; Wielgus 1976). In *Callinectes sapidus* (Cronin 1947), *Portunus sanguinolentus* (Ryan 1967), *Paratelphusa masoniana* (Vasisht and Relan 1971) and *Scylla serata* (Gupta and Chatterji 1976) testes have several lobes and their seminiferous lobules or tubules open into a branched (Cronin 1947) or unbranched seminiferous duct (Ryan 1967). This duct continues posteriorly as vas deferens. In *P. koolooense* the testes are not lobulated and contain numerous seminiferous tubules having germ cells at various stages of spermatogenesis. The tubules become narrower towards posterior side of the testis, where their germ cell area is reduced or absent. These tubules appear to open directly into the vas deferens.

Binford (1913) and Gupta and Chatterji (1976) observed the presence of both spermatocytes and spermatids in the same tubule. Cronin (1947) and Gupta and Chatterji (1976) found that all the spermatocytes of a tubule occur at the same stage of differentiation. In *P. koolooense* the meiotic division occurs more or less synchronously, as the individual maturing tubule contains either spermatids or only one type of spermatocytes which occur at approximately the same stage of differentiation.

The decapod sperm is bicentrosomal or tricentrosomal and aflagellated consisting of a head or nucleus and a vesicle variously described as primary vesicle (Fasten 1926), mitochondrial vesicle (Nath 1932 ; Dhillon 1966) or acrosomal vesicle (Brown 1966 ; Langreth 1969). In brachyura having bicentrosomal sperm, the vesicle contains an axial filament (Nath 1932) or acrosomal tubule (Brown

1966 ; Langreth 1969) which extends up to the distal centrosome (Nath 1932 ; Dhillon 1966). In *P. koolooense* also the sperm is aflagellate consisting of a head and a mitochondrial vesicle which encloses an axial filament and distal centrosome. When stained with PAS all the cells except spermatids and sperms were found negative to this stain. In sperms, the head and mitochondrial vesicle are PAS positive and the axial filament is PAS negative, as also observed by Brown (1966); Dhillon (1966) and Langreth (1969) on other species of Decapoda.

There is paucity of information regarding the origin of new crop of germ cells in crustacean testes. A few primary spermatogonial cells (Binford 1913) or residual spermatogonia (Aoto 1952) which remained undifferentiated till the spermatogenesis is over, undergo divisions shortly after spermiation so as to produce a new batch of secondary spermatogonia. In *P. koolooense* also some undifferentiated or resting spermatogonia are found throughout the year. It appears that after spermiation the new crop of germ cells is supplied by the division of such existing germ cells.

The testes of *P. koolooense* undergo seasonal histomorphological changes associated with change in testicular weight. The measurement of male gonad index revealed two types of spawning patterns among decapods. In continuous breeders like *Penaeus indicus* (Subrahmanyam 1963) and *Portunus pelagicus* (Rahman 1967), the male gonad index was found constant throughout the year whereas in *Charybdis variegata* (Chandran 1968) and *Barytelphusa cunicularis* (Diwan and Nagabhushanam 1974) which breed discontinuously, definite peaks in gonad index were observed. Histological studies revealed the presence of both continuous (Baker and Rosof 1927 ; Spalding 1942 ; Black 1966 ; Ryan 1967 ; Haley 1973) and discontinuous spermatogenetic cycle (Black 1966 ; Chiba and Honma 1972 ; Wielgus 1976) in crustaceans. Such variation in the testicular activity may be due to the genetic differences and the local ecological conditions.

P. koolooense shows discontinuous spermatogenetic cycle. Spermatogenesis begins during January–February, progresses slowly through March, reaching a peak in April or May. However, all the tubules do not mature at the same time, as both maturing and mature tubules were seen in the same section. The mature tubules become filled with sperms whereas maturing tubules consist of dividing spermatocytes or spermatids which produce sperms a little later. This results in the production of sperms in successive waves and spermiation starts before all the tubules are fully packed with sperms. The mature tubules undergo spermiation in May or June. Tubules soon after evacuation of sperms undergo spermatogonial proliferation. In other tubules of the same section of testis, meiosis is still continued. The production of sperms in successive waves during breeding season indicates that one male crab attempts to copulate more than once in a single breeding season. This seems to be advantageous since the fertilization is internal and that the number of males is comparatively fewer than females in a population of *P. koolooense*.

Diwan and Nagabhushanam (1974) reported long resting phase in the reproductive cycle of *Barytelphusa cunicularis*. In *P. koolooense* the spermatogenesis slows down gradually from July onwards and almost ceases by November. The testes enter a brief period of rest during December. In *Pachygrapsus crassipus* also spermatogenesis continues for a longer period during post-spawning period as a result the recovery phase takes place gradually (Chiba and Honma 1972).

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References

- Aoto T 1952 Sexual phases in prawn, *Pandulus kessleri* (Zern), with special reference to the reversal of sex; *J. Fac. Sci. Hokkaido Univ.* **11** 1-20
- Baker R C and Rosof J A 1927 Spermatogenesis in *Branchipus vernalis*. Part I. The testes and spermatogonial division; *Ohio J. Sci.* **27** 175-186
- Binford R 1913 The germ cells and process of fertilization in the crab, *Menippe mercenaria*; *J. Morphol.* **24** 147-202
- Black J B 1966 Cyclic male reproductive activities in dwarf crayfishes, *Cambarellus shufeldtii* (Faxon) and *Cambarellus puer* (Hbbs.); *Trans. Am. Micro. Sci.* **85** 214-232
- Brown G G 1966 Ultrastructural studies of sperm morphology and sperm-egg interaction in decapod, *Callinectes sapidus*; *J. Ultrastr. Res.* **14** 425-440
- Chandran M R 1968 Studies on marine crab, *Charybdis variegata*. I. Reproductive and nutritional cycle in relation to breeding periodicities; *Proc. Indian Acad. Sci.* **B67** 215-223
- Chiba A and Honma Y 1971 Studies on gonad maturity in some invertebrates. II. Structure of reproductive organs of the lined shore crab; *Bull. Jpn. Soc. Sci. Fish.* **37** 699-706
- Chiba A and Honma Y 1972 Studies on the gonad maturity in some marine invertebrates. VI. Seasonal changes in testes of the lined shore crab; *Bull. Jpn. Soc. Sci. Fish.* **38** 317-322
- Cronin L E 1947 Anatomy and histology of the male reproductive system of *Callinectes sapidus* (Rathbun); *J. Morphol.* **81** 209-233
- Dhillon B 1966 Cytochemical changes in cell organelles in spermatogenesis of decapod crustacea; *Res. Bull. Punjab Univ.* **17** 55-56
- Diwan A D and Nagabhushanam R 1974 Reproductive cycle and biochemical changes in the gonad of freshwater crab, *Barytelphusa cunicularis* (Westwood); *Indian J. Fish.* **21** 164-176.
- Fasten N 1926 Spermatogenesis of the black clawed crab, *Lophopanopeus bellus*; *Biol. Bull.* (Woodshole) **1** 277-293
- Giese A C 1959 Annual reproductive cycle of marine invertebrates. Comparative physiology; *Ann. Rev. Physiol.* **21** 547-576
- Gupta S R and Chatterji N B 1976 Anatomical observations of the internal male reproductive organs of *Scylla serrata*; *Indian J. Physiol. Allied Sci.* **30** 34-42
- Haley S R 1973 On the use of morphometric data as a guide to reproductive maturity in the ghost crab, *Ocypode ceratophthalmus* (Pallas) (Brachyura, Ocypodidae); *Pac. Sci.* **27** 350-362
- Iyer Muthuswamy M S 1933 Spermatogenesis of *Paratelphusa hydrodromous* with a note on oogenesis; *J. Mysore Univ.* **7** 206-232
- King J E 1948 A study of the reproductive organs of common marine shrimp, *Penaeus setiferus* (Linn.); *Biol. Bull.* **94** 244-250
- Langreth S G 1969 Spermiogenesis in cancer crabs; *J. Cell. Biol.* **43** 575-603
- Nath V 1932 The spermatid and sperm of the crab, *Paratelphusa spinigera*; *Quart. J. Micro. Sci.* **75** 543-556
- Nath V 1965 *Animal gametes (Male)* (Bombay: Asia Publishing House) pp. 78-93
- Rahman A A 1967 Reproductive and nutritional cycles of the crab, *Portunus pelagicus* (Linn.) of Madras coast; *Proc. Indian Acad. Sci.* **B65** 76-82

- Ryan E P 1967 Structure and function of the reproductive system of the crab, *Portunus sanguinolentus* (Horb.). I. The male system; *Proc. Symp. Crustacea Ernakulum, Mar. Biol. Assoc. India* 2 506-521
- Spalding J S 1942 The nature and formation of spermatophore and sperm plug in *Carcinus maenas*; *Quart. J. Micro. Sci.* 183 399-422
- Subrahmanyam C B 1963 A note on the reproductive cycle of the prawn, *Penaeus indicus* (N. Edw.) of Madras coast; *Curr. Sci.* 4 165-166
- Vasisht H S and Relan U 1971 Anatomy of *Paratelphusa masoniana* (Hend.). V. Reproductive system; *Res. Bull. Punjab Univ.* 22 13-18
- *Wielgus S E 1976 Morphological and histological changes in the male gonad of the American crayfish, *Orconectes limosus* (Refinesque) in annual cycle; *Acta Biol.* 19 87-105
- Wolfe A F 1971 A histological and histochemical study of the male reproductive system of *Artemia* (Crustacea, Branchiopoda); *J. Morphol.* 135 51-70

* Not consulted in original.

Seasonal changes in the ovary of a freshwater crab, *Potamon koolooense* (Rathbun)

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Abstract. The ovaries of *P. koolooense* which are paired H-shaped structures undergo seasonal morphometric and histological changes. A minute oviduct leads into the seminal receptacle which receives sperms during breeding season. Oogonia and young oocytes develop in the germinal zone, present in the centre of the ovary. The resting or residual oogonia which occur throughout the year divide shortly after ovulation and supply new crop of germ cells for the next breeding season. Five maturational stages of ova have been described on the basis of changes that occur in their nuclei and cytoplasm. They are oogonium, premeiotic oocyte, previtellogenic oocyte, vitellogenic oocyte and the ripe ovum. Spawning occurs during May or June. The weight of the ovaries, gonad index and ova diameter were minimum in June and reached a maximum value in April.

Keywords. *Potamon*; ovarian histology; ovarian cycle; vitellogenesis.

1. Introduction

The structure of female reproductive organs has been described in some species of crabs (Weitzman 1966; Rouquette 1970; Chiba and Honma 1971; Laulier and Demeusy 1974). The process of yolk formation differs in various species of crustaceans (Harvey 1929; Bhatia and Nath 1931; Hinsch and Cone 1969; Hinch 1970). Most of the studies on the female reproductive cycle are based on the morphometric characters of the ovaries only. Nevertheless, relatively little emphasis has been laid on the seasonal histological changes in the ovaries of the crabs and other decapods (Weitzman 1966; Chiba and Honma 1972; Laulier and Demeusy 1974; Badawi 1975; Goldstein and Lauria 1975; Rao *et al* 1981). In the present investigation the structure and seasonal histomorphological changes in the ovaries of a hill stream crab, *Potamon koolooense* were studied.

2. Material and methods

15 to 20 live specimens of adult female *P. koolooense* (carapace width 3.8 to 4.5 cm) were collected locally from a stream each month during 1976-78. The

weight of each specimen was recorded immediately before dissection. Ovaries along with oviducts and seminal receptacles were removed and placed in fixative. Ovarian weight was recorded after fixation. The gonosomatic index (GSI) was calculated using the formula (Giese 1959) :

$$\text{GSI} = \frac{\text{weight of the gonad}}{\text{weight of the animal}} \times 100.$$

For histology, different regions of ovaries were fixed in Bouin's or Helly's fluid. Paraffin sections of 5-6 micra thickness were cut and stained with Delafield's haematoxylin or Mayer's haemalum, using eosin as counterstain. Heidenhain's Azan or Mallory's triple stain was also used. The average oocyte diameter was calculated by measuring rounded oocytes having complete nuclei. The moulting stages of the crabs are not determined in this paper.

3. Observations

3.1. *Morphology of reproductive organs*

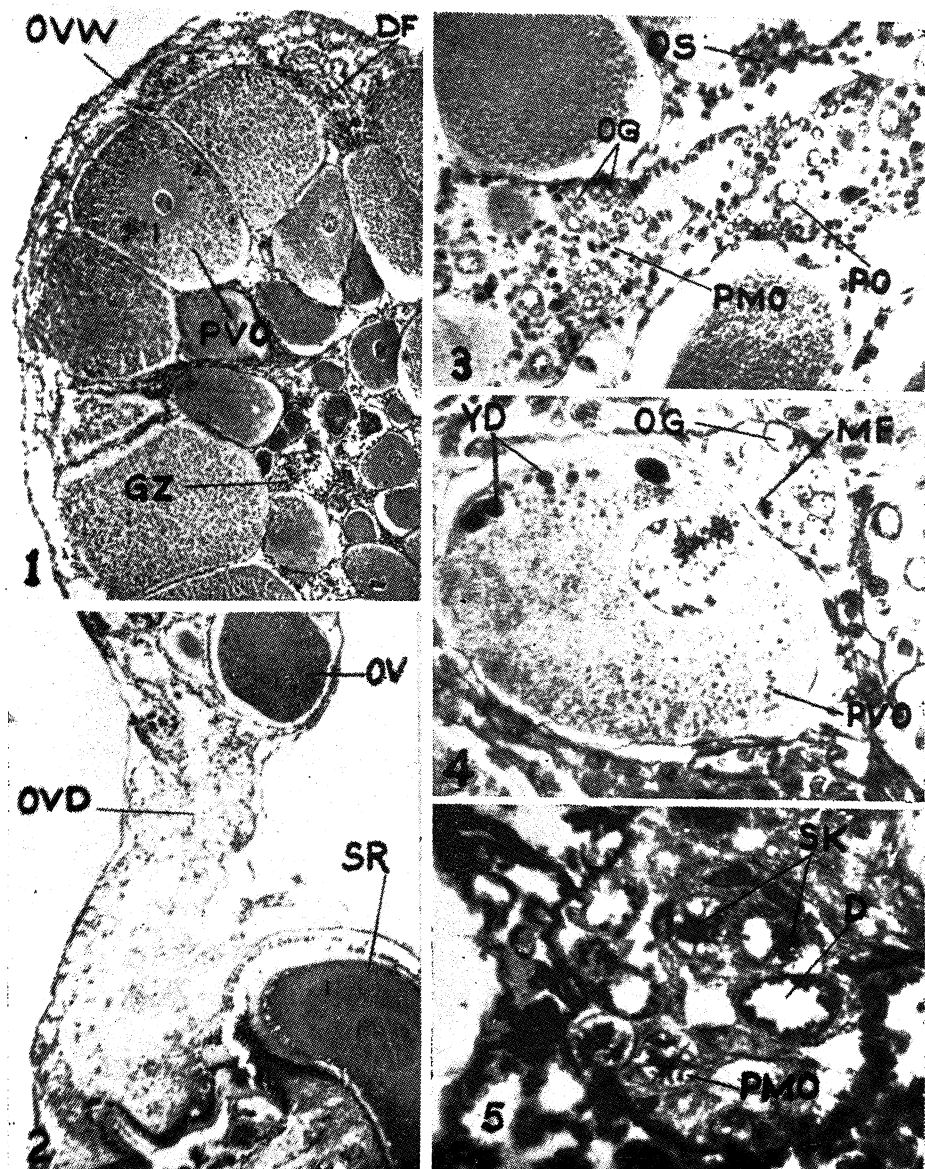
The female reproductive organs include paired ovaries, oviducts and seminal receptacles. Ovaries are elongated 'H'-shaped structures, situated between hypodermis of carapace and the hepatopancreas. Just behind the pyloric stomach, the two ovaries are connected together by a cross connection. Ovary leads into a very minute oviduct. In sections the ovarian wall appears to continue as the oviduct (figure 2), which opens into a large thick walled pouch, the seminal receptacle. Each receptacle leads into a narrow vaginal tube which further opens outside through a small circular gonopore situated on the 6th sternal segment of the cephalothorax. Ovaries show morphological changes associated with their degree of maturity, as reflected in their size, shape, colour and weight.

3.2. *Histology of ovary*

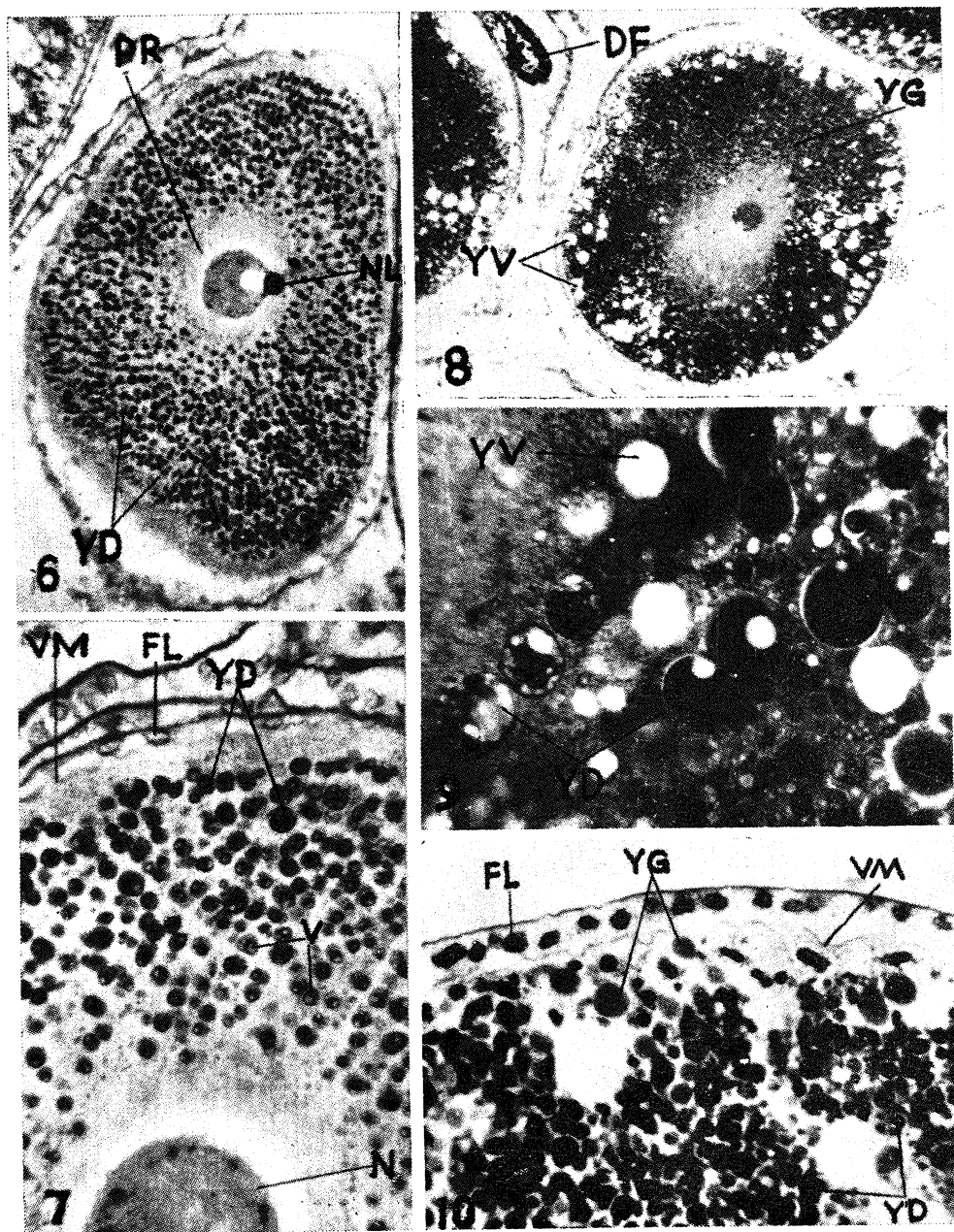
The ovarian wall is continuous with ovarian stroma (figure 1) and is thicker during post-spawning period but becomes thin at maturing and mature stages of the ovary. Ovarian stroma consists of connective tissue, muscle fibres and blood vessels, and is abundant during post-spawning period but greatly reduced in mature ovaries (figure 1). A germinal zone is present all along the centre of the ovary (figures 1, 3). During early phases of maturation, the germinal zone consists of oogonia and young oocytes whereas the developing oocytes are displaced towards outer region of the ovary (figure 1). As maturity advances the germinal zones become greatly reduced consisting of a few residual oogonia, the rest of the ovary is filled with maturing oocytes.

3.3. *Stages of developing ova*

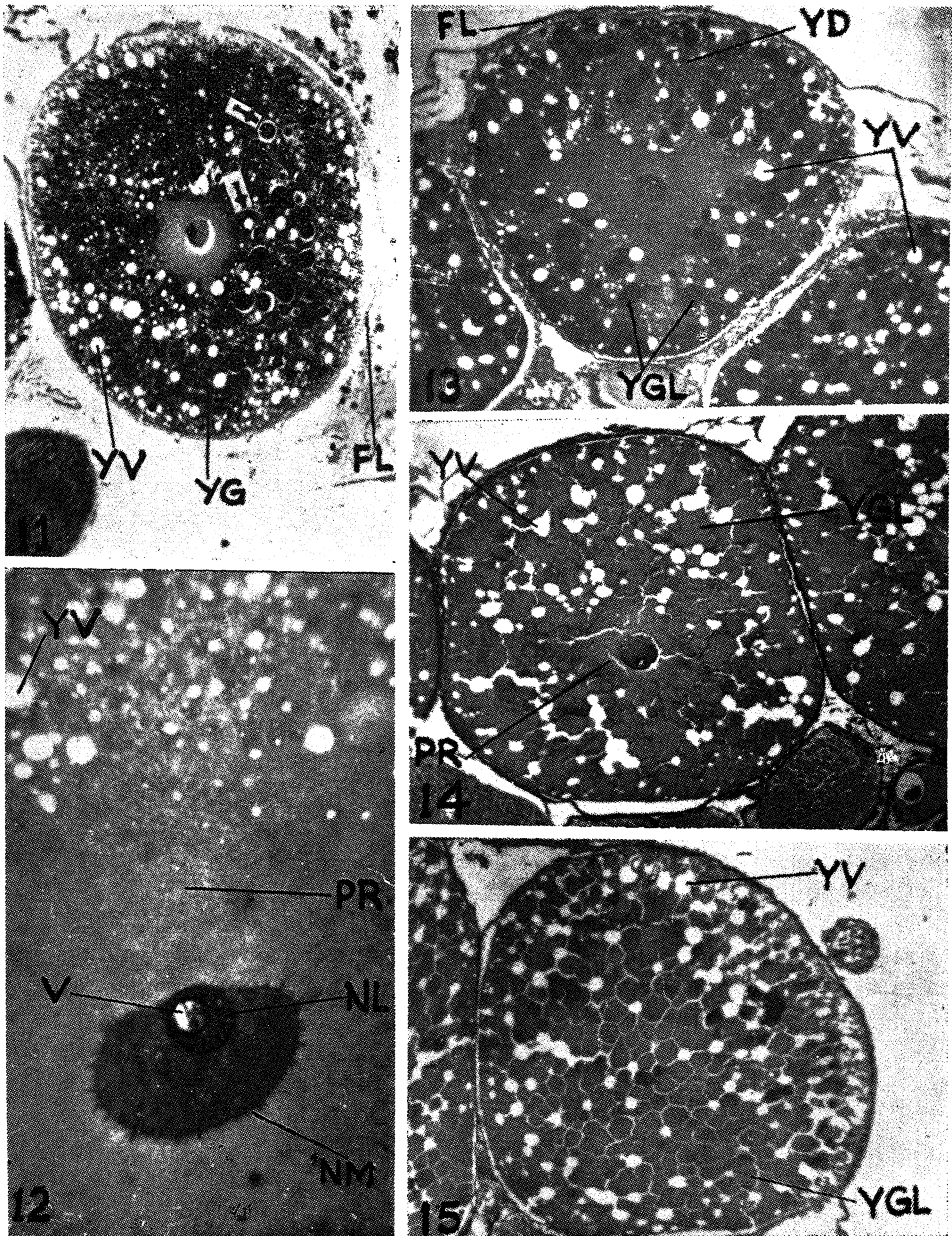
The oogonium passes through different maturational stages before it becomes the ripe ovum. This process involves changes in the nucleus and cytoplasm. According to the classification of Raven (1961) and Laulier (1974), the following developmental stages have been observed :



Figures 1-5. 1. Structure of ovary in transverse section, June specimen $\times 40$. 2. Section passing through oviduct (OVD) $\times 40$. 3. L.S. of ovary showing germinal zone (GZ) $\times 140$. 4. Oogonium (OG) showing mitotic figure (MF), yolk droplets (YD) appear in peripheral ooplasm of primary vitellogenic oocyte (PVO) $\times 380$. 5. Showing premitotic oocyte in germinal zone $\times 380$. (DF, discharge follicle; GZ, germinal zone; OG, oogonia; OS, ovarian stroma; OV, ovary; OVW, ovarian wall; PMO, premitotic oocytes; PO, previtellogenic oocytes; PVO, primary vitellogenic oocytes; SK, synzezeis knot; SR, seminal receptacle).



Figures 6-10. 6. Primary vitellogenic oocyte showing yolk droplets (YD) extended towards perinuclear region (PR). Displacement of nucleolus is due to mechanical disturbance during section cutting $\times 140$. 7. An enlarged portion of above showing vacuoles (V) inside yolk droplets (YD) $\times 380$. 8. Yolk granules (YG) and yolk vesicles (YV) in periphery of secondary vitellogenic oocyte $\times 70$. 9. Vacuolation of yolk droplets (YD) to form yolk vesicles (YV) $\times 380$. 10. Portion of secondary vitellogenic oocyte showing light yolk granules (YG), dark yolk droplets (YD) and yolk vesicles (YV) $\times 380$, (DF, discharged follicle; FL, follicular layer; N, nucleus, VM, vitelline membrane.)



Figures 11-15. 11. Secondary vitellogenic oocyte showing vacuolation of yolk droplets (arrow). Perinuclear region is yolkless $\times 40$. 12. Showing vacuolation (v) and eccentric position of nucleolus (NL) $\times 380$. 13. Secondary vitellogenic oocyte showing yolk globules (YGL) and yolk vesicles (YV). Yolk droplets (YD) are in the background $\times 60$. 14. Tertiary vitellogenic oocyte filled with yolk globules (YGL) and yolk vesicles (YV). Perinuclear region (PR) is occupied by yolk $\times 60$. 15. Ripe ovum filled with yolk globules (YGL) and yolk vesicles (YV) $\times 40$. (FL, follicular layer; NM, nuclear membrane; PR, perinuclear region; YG, yolk granules; YV, yolk vesicles).

3.3a. *Oogonium* (figures 3, 4, 5) : A few primary or residual oogonia which occur throughout the year in the germinal zone, divide mitotically (figure 4) shortly after ovulation and give rise to additional oogonia to provide for further growth of ovary. Oogonium is a small spherical cell with a pale nucleus and thin rim of poorly basophilic cytoplasm. Oogonia develop into premeiotic oocytes but a few remain undifferentiated (residual oogonia) till the ovulation.

3.3b. *Premeiotic oocyte* (figures 3, 5) : The oogonia which enter into prophase activities are termed as premeiotic oocytes or primary oocytes. The chromosomes condense at one side of the nucleoplasm and form synzezeis knot (figure 5) which corresponds with zygotene or synapsis stage of meiotic prophase. At the final developmental stage (diakinesis) the chromatin appears in the form of discrete clumps lying close to nuclear wall (figure 5) and the nucleolus is not visible. The oocyte measures 20 micra in diameter.

3.3c. *Previtellogenic oocyte* (figure 3) : As the premeiotic activities come to an end, the nucleus increases in volume and oocyte acquires a large amount of basophilic cytoplasm. The nucleus appears vesicular containing peripherally arranged chromatin clumps and a centrally placed nucleolus which appear solids and stain blue-black with haematoxylin or red with Mallory's triple or Azan stains. The yolk formation has not yet begun and oocyte attains a diameter of 95 micra.

3.3d. *Vitellogenic oocyte* : The oocyte now enters a synthetic or vegetative phase resulting in the formation of yolk. The nucleus, nucleolus and ooplasm undergo marked changes in their cytology as described below :

(a) Primary vitellogenic oocyte (figures 4, 6, 7) : Further increase in amount of basophilic ooplasm and the volume of nucleus and nucleolus accompanies the appearance of few small yolk droplets in peripheral ooplasm (figure 4). The yolk droplets stain purple to black with haematoxylin or blue with Mallory's triple or Azan stains. Chromatin clumps become finely granular arranged in a network in nucleoplasm. Nucleus is solid and central in position. A thin layer of follicular cells forms around the oocyte (figures 6, 7). Further increase in oocyte diameter is accompanied with increase in amount of yolk droplets which progressively extends towards the yolkless and homogeneous perinuclear region (figures 6, 7). The yolk droplets swell and minute unstainable vacuoles begin to appear in them (figure 7). Average diameter of oocyte is 250 micra.

(b) Secondary vitellogenic oocyte (figures 8, 9, 11, 13) : The oocyte shows a rapid growth and the unstainable vacuoles in yolk droplets increase in number and size (figures 8, 11). This results in marked decrease in stainable contents of yolk droplets which thus appear reticulated or spongy. Vacuoles fuse with each other and ultimately form large unstainable yolk vesicles, initially in peripheral ooplasm but later on in other regions (figures 9, 11, 13). This is followed by the appearance of small eosinophilic yolk granules in the extravascular ooplasm (figures 9, 11). In Mallory's triple or Azan stains the yolk granules stain red. They increase in size probably by their fusion and thus gives rise to large oval yolk globules

(figure 13). Oolemma and follicular layers are well differentiated (figure 10). While the yolk granules are appearing in the ooplasm, the nucleolus enlarges and becomes eccentric and vacuolated without any change in its tinctorial properties (figures 12, 13). Yolk droplets gradually disappear. Oocytes measure 600 micra in diameter.

(c) Tertiary vitellogenic oocyte (figure 14): With further growth of oocyte, the amount of yolk globules increases. Yolk granules still continue to appear in peripheral ooplasm. Yolk droplets are absent because of their conversion into yolk vesicles. Ooplasm thus becomes entirely acidophilic. Yolk globules become polygonal and occupy most of the ooplasm including the perinuclear region. Nucleolus is eccentric in position and poorly stainable due to its extensive vacualization. Follicular layer is stretched to a thin membrane and its nuclei become spindle shaped, probably due to the turgidity of oocyte. Vitelline membrane is distinct. Oocyte is 950 micra in diameter.

3.3e. *Ripe ovum* (figure 15): It is largest in size measuring upto 1400 micra in diameter. Ooplasm is heavily impregnated with large yolk globules and yolk vesicles.

3.4. *Seasonal changes in ovaries*

The annual ovarian cycle can be conveniently divided into following four stages on the basis of histomorphological features of ovaries :

3.4a. *Spawning and spent stage (May-June)*: During this period females carry their spawn attached onto their pleopods. Ovaries are small, smooth and cream coloured. Seminal receptacles contain sperms. Discharged follicles are found in stroma, indicating that the spawning has taken place (figure 1). Ovarian wall becomes thick and the germinal zone consists of numerous oogonia, premeiotic and previtellogenic oocytes (figures 1, 3). A few primary vitellogenic oocytes also develop at peripheral region of ovary.

3.4b. *Early maturing stage (July-October)*: Ovaries increase in size and are coloured deep yellow. Externally they appear granular owing to the preponderance of primary and secondary vitellogenic oocytes in them. Germinal zone contains a few residual oogonia and previtellogenic oocytes.

3.4c. *Advanced maturing stage (November-February)*: Ovaries become enlarged, convoluted and orange in colour. Large orange ova bulge out on the surface of ovaries. Tertiary vitellogenic oocytes are commonly present but a few secondary ones also occur. Premeiotic and primary vitellogenic oocytes are rare in the ovaries.

3.4d. *Mature stage (March-April)*: Ovaries attain a maximum size and deep orange mature ova are visible through the thin ovarian wall. The gonosomatic index is minimum in June and reaches a maximum value in April and is in conformity with the average ova diameter (figure 16).

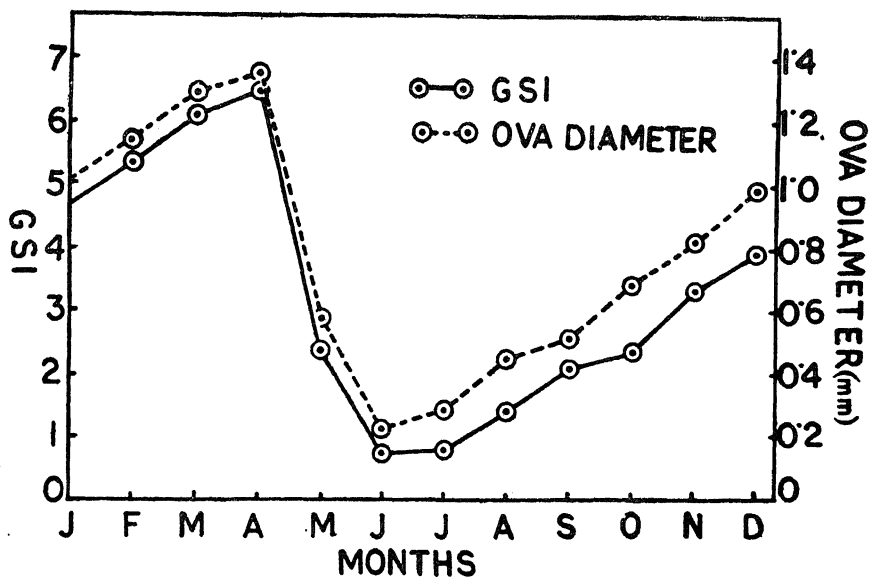


Figure 16. Seasonal changes in the gonosomatic index (GSI) and ova diameter.

4. Discussion

The female reproductive organs of *P. koolooense* are built on the same general plan as observed in other crabs by Hartnoll (1968), Vasisht and Relan (1971) and Chiba and Honma (1971). Several decapods possess a germinal zone in the centre of ovary (Kessel 1968 ; Hinsch and Cone 1969 ; Rouquette 1970 ; Laulier and Demeusy 1974 ; Rao *et al* 1981) whereas in others the germinal zone is peripheral (Cronin 1942 ; King 1948) or is in the form of nests of germ cells distributed throughout the ovary (Weitzman 1966). In *P. koolooense* the germinal zone occurs at the centre of ovary.

Little is known about the origin of new crop of germ cells in adult decapods. It is generally agreed that the new crop of germ cells arise by division of existing oogonia. In *Gecarcinus lateralis* (Weitzman 1966) and *Pachygrapsus marmoratus* (Rouquette 1970) oogonial mitosis occurs throughout the year. In *P. koolooense* the resting oogonia or residual oogonia occur throughout the year but they divide shortly after ovulation and produce a new drop of oogonial cells for further growth of ovaries, as observed by Aoto (1952) and Laulier and Demeusy (1974) in other decapods.

The process of yolk formation varies considerably in different decapods. The yolk vesicles and yolk globules in the oocyte of *P. koolooense* correspond with the fatty yolk vacuoles and proteinous yolk bodies of *Carcinus maenas* (Harvey 1929), *Palaemon lamarrei* and *Paratelson spinigera* (Bhatia and Nath 1931) and *Paratelson hydromedusae* (Vasisht and Relan 1971). Bhatia and Nath (1931) observed that the fatty yolk vacuoles appear initially at the peripheral ooplasm while the protein yolk bodies near perinuclear region. In shrimp, *Chirocephalus bundyi* (Linder 1959) the fatty yolk droplets and proteinous yolk granules first appear in the central region of ooplasm. In *P. koolooense* both yolk vesicles

and yolk granules first appear at peripheral ooplasm and then extend progressively towards perinuclear region as has also been observed by Harvey (1929) in *Carcinus maenas*.

Besides the structural changes, the yolk bodies and nucleolus in some crabs undergo parallel changes in their cytochemical nature and staining affinity (Otsu 1963 ; Carmignani *et al* 1973). In *P. koolooense*, neither the yolk globules nor the nucleolus undergo changes in their staining reactions. The source of yolk material differs in various species of crustaceans according to several authors. Generally the yolk is formed from both extra oocyte sources (perinuclear endoplasmic reticulum and golgi bodies in collaboration with nucleolar extrusions (Kessel 1968 ; Hinsch and Cone 1969 ; Hinsch 1970 ; Dhainaut and Leersynder 1976) and extra oocyte sources (yolk precursors are incorporated from haemolymph into ooplasm) by diffusion through follicular cell layer (Linder 1959 ; Beams and Kessel 1963) or by micropinocytosis at oocyte surface (Hinsch and Cone 1969 ; Dhainaut and Leersynder 1976).

The nucleolus during vitellogenesis undergo progressive increase in size and shows vacuolation (Harvey 1929 ; Hinsch 1970 ; Dhainaut and Leersynder 1976). The nucleolar extrusions or granules which pass into ooplasm are believed to take part in yolk formation (endogenous yolk) in crabs (Harvey 1929 ; Bhatia and Nath 1931 ; Hinsch 1970). In *P. koolooense*, neither the nucleolar granules were seen nor the nucleolus was found moved into the ooplasm. Although the increase in the size of nucleolus, its eccentric position and progressive vacuolization during vitellogenesis in this species indicates that it has some role in yolk formation, nothing can be said about its functions in the absence of direct evidence.

Seasonal morphometric studies revealed that the breeding cycle in crabs and other decapods varies widely, even in species having close taxonomic relationships or similar ecological niches, *i.e.*, (i) continuous breeder around the year (Booolootian *et al* 1959 ; Knudsen 1964 ; Rahman 1967 ; Badawi 1975), (ii) Seasonal breeders having one spawning season (Booolootian *et al* 1959 ; Hartnoll 1963 ; Otsu 1963 ; Knudsen 1964 ; Diwan and Nagabhushanam 1974 ; Bomirski and Klek 1974 ; Badawi 1975) or two distinct spawning seasons (Knudsen 1964 ; Chandran 1968 ; Adiyodi 1968, Goldstein and Lauria 1975). *P. koolooense* is a seasonal breeder, as all the oocytes in the ovaries become fully mature at the onset of breeding season and are laid simultaneously during May or June, followed by the next ovarian cycle. The gonad index begins to decline in May, reaching a minimum level in June and increases slowly during the following months with a peak in April (figure 16). Such variations in the breeding season among decapods may be due to the genetic differences and the local ecological conditions.

Studies on the seasonal histological changes in the ovaries revealed that in post spawning period the vitellogenesis takes place late and is preceded either by a long resting stage (Weitzman 1966 ; Diwan and Nagabhushanam 1974) or by recovery or previtellogenic stages (Chiba and Honma 1972 ; Badawi 1975). In biannual breeders, the vitellogenesis of second ovarian cycle begins soon after the completion of the preceding cycle while in first ovarian cycle the vitellogenesis is preceded either by long resting phase (Adiyodi 1968) or a brief resting stage

(Bomirski and Klek 1974). In *P. koolooense* vitellogenesis occurs shortly after spawning and it appears that the resting stage in this species is of very short duration probably of a few days only.

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References

- Adiyodi R G 1968 On the reproduction and molting in the crab, *Paratelphusa hydrodromous*; *Physiol. Zool.* **41** 204-209
- Aoto T 1952 Sexual phases in the prawn, *Pandulus kessleri* (Zern.) with special reference to the reversal of sex; *J. Fac. Sci. Hokkaido Univ.* **11** 1-20
- Badawi H K 1975 On maturation and spawning in some penaeid prawns of Arabian Gulf; *Mar. Biol. (Berlin)* **32** 1-6
- Beams H W and Kessel R G 1963 Electron microscopic studies on developing crayfish oocytes with special reference to the origin of yolk; *J. Cell Biol.* **18** 621-649
- Bhatia D R and Nath V 1931 Studies on the origin of yolk. VI. The crustacean oogenesis; *Quart. J. Micro. Sci.* **74** 669-701
- Bomirski A and Klek E 1974 Action of eyestalks on the ovary in *Rhithropanopeus harrisi* and *Craxon craxon* (Crustacea, Decapoda); *Mar. Biol.* **24** 329-337
- Booolootian R A, Giese A C, Farmanfarman A and Tucker J 1959 Reproductive cycle of five west coast crabs; *Physiol. Zool.* **32** 213-220
- Carmignani M, Albanese P, Bolognari A and Zaccove G 1973 Morphological study on the yolk globules in the growing oocyte of *Maja verrucosa* (Edw.) (Crustacea, Brachyura); *Acta Histochem.* **46** 195-201
- Chandrian M R 1968 Studies on marine crab, *Charybdis variegata*. I. Reproductive and nutritional cycle in relation to breeding periodicities; *Proc. Indian Acad. Sci.* **B67** 215-223
- Chiba A and Honma Y 1971 Studies on gonad maturity in some marine invertebrates. II. Structure of reproductive organs of the lined shore crab; *Bull. Jpn. Soc. Sci. Fish.* **37** 699-706
- Chiba A and Honma Y 1972 Studies on gonad maturity in some marine invertebrates. VII. Seasonal changes in the ovary of the lined shore crab; *Bull. Jpn. Soc. Sci. Fish.* **38** 323-329
- *Cronin L E 1942 A histological study of the development of ovary and accessory reproductive organs in the blue crab, *Callinectes sapidus* (Rathbun); *M.S. Thesis Univ. Maryland*
- Dhainaut A and Leersnyder De M 1976 Cytochemical study of the oocyte development in crab, *Eriocheir sinensis*. I. Natural oogenesis; *Arch. Biol.* **87** 261-282
- Diwan A D and Nagabhushanam R 1974 Reproductive cycle and biochemical changes in gonads of freshwater crab, *Barytelphusa cunicularis* (Westwood); *Indian J. Fish.* **21** 164-176
- Giese A C 1959 Annual reproductive cycle of marine invertebrates; *Ann. Rev. Cytol.* **21** 547-576
- Goldstein B and Lauria L 1975 Cycle of sexual maturation and preliminary studies on spawning of the freshwater shrimp, *Palaemonetes argentinus*. I. Female; *Physis. Agnas Cont. Org. Sect.* **B33** 165-176
- Hartnoll R G 1963 The biology of manx spider crabs; *Proc. Zool. Soc. London* **141** 423-496
- Hartnoll R G 1968 Morphology of the genital ducts in female crabs; *J. Linn. Soc. (Zool.)* **47** 279-300
- Harvey L A 1929 The oogenesis of *Carcinus maenas* (Penn.) with special reference to yolk formation; *Trans. Roy. Soc. Edinburgh* **56** 157-176

- Hinsch G W 1970 Possible role of intranuclear membranes in nuclear cytoplasmic exchange in spider crab oocytes; *J. Cell Biol.* **47** 531-535
- Hinsch G W and Cone M V 1969 Ultrastructural observations of the vitellogenesis in spider crab, *Libinia emarginata*; *J. Cell Biol.* **40** 336-342
- Kessel R G 1968 Mechanism of protein yolk synthesis and deposition in crustacean oocyte; *Z. Zellforsch.* **89** 17-38
- King J E 1948 A study of the reproductive organs of the common marine shrimp, *Penaeus setiferous* (Linn.); *Biol. Bull.* **94** 244-250
- Knudsen J W 1964 Observations of the reproductive cycle and ecology of the common brachyura and crab like anomura of Puget Sound coast, Washington; *Pac. Sci.* **18** 3-33
- Laulier M 1974 Caracteres cytologique de la cellule sexuelle femelle du crabe, *Carcinus maenas* L. au cours de la gametogenese; *Cah. Biol. Mar.* **15** 159-167
- Laulier M and Demeusy N 1974 Etude histologique du fonctionnement ovarien au cours d'une maturation de ponte chez le crabe, *Carcinus maenas* L. (Crustacea, Decapode); *Cah. Biol. Mar.* **15** 343-350
- Linder H J 1959 Studies on freshwater fairy shrimp, *Chirocephalus bundyi* (Forbes). I. Structure and histochemistry of ovary and accessory reproductive tissue; *J. Morphol.* **104** 1-60
- Otsu T 1963 Bihormonal control of sexual cycle in the freshwater crab, *Potamon dehaani*; *Embryologica* **8** 1-20
- Rahman A A 1967 Reproductive and nutritional cycle of the crab, *Portunus pelagicus* (Linnaeus) (Decapode, Brachyura) of Madras coast; *Proc. Indian Acad. Sci.* **B65** 76-82
- Rao Ch Narasimha, Shakuntala K and Reddy S R 1981 Molt-reproduction relationship in the freshwater prawn, *Machrobrachium lancesteri* (de Man); *Proc. Indian Acad. Sci.* **90** 39-52
- Raven C P 1961 *Oogenesis: The storage of developmental information* (London and New York: Pergamon Press)
- Rouquette M 1970 Etude du tissu ovarien chez le crabe *Pachygrapsus marmoratus* (Fabricius) Premiers resultats concernant les role de la temprature et des pedoncles oculaires; *Bull. Soc. Zool. France* **95** 233-240
- Vasisht H S and Relan U 1971 Anatomy of *Paratelphusa masoniana* (Henderson). V. Reproductive system; *Res. Bull. Punjab Univ.* **22** 13-18
- Weitzman M C 1966 Oogenesis in tropical land crab, *Gecarcinus lateralis* (Fremerville); *Z. Zellforsch.* **75** 109-119

* Not consulted in original.

Evaluation of warfarin against *Tatera indica* and *Meriones hurrianæ*

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Abstract. Warfarin was evaluated in laboratory against Indian gerbil, *Tatera indica* and desert gerbil, *Meriones hurrianæ*. Chronic LD_{50} for the two species was found to be 4×19.1 and 4×15.9 mg/kg respectively. Feeding for 14 days on 0.025% warfarin treated bait provided complete kill in the gerbils but the poisoned bait was less palatable than the plain bait. A period of 18 and 19 days feeding on 0.025% warfarin bait was found suitable to detect resistance to warfarin among *T. indica* and *M. hurrianæ* respectively.

Keywords. *T. indica*; *M. hurrianæ*; oral toxicity; no-choice tests; base-line susceptibility; palatability; warfarin.

1. Introduction

The Indian gerbil, *Tatera indica* Hardwicke and the desert gerbil, *Meriones hurrianæ* (Jerdon) are dominant rodent species in the Indian desert and inflict severe damage to crops and grasslands (Barnett and Prakash 1975). Since they induce bait shyness after a single exposure of zinc phosphide (Prakash and Jain 1971) the need to evaluate other rodenticides as alternative poisons for their control has arisen. The present study was, therefore, undertaken to evaluate warfarin [3-(1-phenylethyl-2 acetyl) 4-hydroxy-coumarin] against *T. indica* and *M. hurrianæ*.

2. Material and methods

The gerbils were captured from fields around Jodhpur (Lat. $26^{\circ} 18' N$; Long. $73^{\circ} 1' E$). They were sexed, weighed and caged individually for 3 weeks for acclimatization and were fed on bajra (*Pennisetum typhoides*) and jowar (*Sorghum vulgare*). Average body weights of *T. indica* and *M. hurrianæ* (g; mean \pm SE) were 124.16 ± 5.73 and 62.44 ± 3.62 respectively. Each of the four doses (5.0, 15.0, 25.0 and 50.0 mg/kg) of technical warfarin of 98% purity was administered by oral tube for four consecutive days to calculate the chronic LD_{50} . No-choice and choice feeding trials were conducted using 0.0125% and 0.025% warfarin-

treated bajra grains. The former trials were conducted for different lengths of feeding periods. In choice tests an alternative unpoisoned bait was also provided to the gerbils. The trials were conducted as recommended by WHO (1976) and the LD_{50} 's, lethal feeding periods (LFP_{50} and LFP_{95}) and their 95% confidence limits were calculated by probit analysis (Finney 1971).

3. Results

Sex difference in the mortality was not observed in any of the trials and hence combined sex mortality data were analysed.

3.1. Oral toxicity

Chronic LD_{50} and 95% confidence limits for *T. indica* and *M. hurrianae* are 4×19.1 (13.8–27.61) and 4×15.9 (11.0–24.0) mg/kg respectively. Slopes of the probit regression line with respect to two species are $1.48 \pm S.E. 0.12$ and 1.61 ± 0.12 respectively.

3.2. No-choice tests

In no-choice feeding tests complete kill was observed with 14 days feeding on 0.0125 and 0.025% warfarin treated bait in both the species (table 1) except that with the former concentration one *T. indica* survived.

In both the gerbils, *T. indica* and *M. hurrianae*, mortality started from day 4 and 5 and lasted upto days 18 and 16 respectively and maximum kill occurred between 5 to 10 days (table 1). Bait intake in no-choice test was fairly high upto 6–7 days after which it declined possibly due to the development of the symptoms of anticoagulant poisoning.

3.3. Base-line susceptibility

Table 2 gives the lethal feeding periods (LFP_{50} and LFP_{95}), their 95% confidence limits and slopes of the probit regression lines. The slope of the probit regression line and LFP_{50} does not differ significantly between the sexes and concentration but significant difference was found between species ($P < 0.02$) with respect to 0.025% concentration (table 2) which indicates that *M. hurrianae* is more susceptible to warfarin than *T. indica*.

3.4. Acceptability of poisoned bait

Poisoned bait was less palatable than the plain bait (table 3). The difference was not significant between the two concentrations in both the species. However, with both the concentrations the intake of poisoned bait by *M. hurrianae* was significantly more ($P < 0.01$) than *T. indica* (table 3) and hence the mortality was higher in the former species.

Table 1. Mortality in *T. indica* and *M. hurrianae* feeding on warfarin-treated pearl millet in no-choice tests.

Feeding period (days)	Conc. of poison (percent)	Anticoagulant consumed (mg/kg), Mean \pm S.E.		Mortality	Days to death	
		Died	Survived		Mean	Range

<i>Tatera indica</i>						
2	0.0125	...	14.36 \pm 0.84	0/10
4		24.48 \pm 3.78	26.06 \pm 2.56	2/10	4.5	4-5
7		42.38 \pm 5.43	46.98 \pm 5.20	6/10	10.0	7-13
10		41.70 \pm .57	65.38	9/10	9.1	5-12
14		39.16 \pm 4.07	81.25	11/12	8.2	5-11
2	0.025	38.65	21.30 \pm 2.17	1/10	18.0	...
4		41.61 \pm 11.23	56.80 \pm 3.17	4/10	5.2	5-6
7		104.16 \pm 5.51	108.83 \pm 6.67	6/12	6.6	4-8
10		72.20 \pm 3.99	60.75	9/10	8.5	7-13
14		86.27 \pm 9.23	...	12/12	8.6	5-14
<i>Meriones hurrianae</i>						
2	0.0125	22.32	19.98 \pm 2.24	1/10	11.0	...
4		28.17 \pm 2.36	22.47 \pm 5.90	4/10	6.7	5-10
7		79.40 \pm 9.43	80.67 \pm 7.19	6/10	7.3	4-11
10		85.45 \pm 14.60	99.95 \pm 2.65	7/10	9.7	6-15
14		96.46 \pm 9.96	...	10/10	10.5	7-15
2	0.025	21.51	30.58 \pm 4.06	1/10	6.0	...
4		47.22 \pm 8.40	57.56 \pm 5.37	5/10	5.6	4-7
7		114.10 \pm 14.95	103.57 \pm 23.45	8/12	8.4	5-12
10		114.41 \pm 21.59	148.83 \pm 16.18	8/10	8.1	5-14
14		173.16 \pm 16.98	...	10/10	11.4	5-16

Table 2. Lethal feeding periods (LFP) for *T. indica* and *M. hurrianae* and their 95% fiducial limits using warfarin.

Species	Conc. of poison (percent)	Slope of the probit regression line (b) \pm S.E.	LFP ₅₀ (days)	LFP ₉₅ (days)
<i>T. indica</i>	0.0125	1.92 \pm 0.10	6.0 (4.4-8.1)	16.6 (8.9-30.9)
	0.025	1.13 \pm 0.08	5.7 (4.2-7.9)	13.2 (10.0-17.4)
<i>M. hurrianae</i>	0.0125	1.81 \pm 0.07	4.6 (3.8-5.7)	13.5 (10.0-18.2)
	0.025	1.89 \pm 0.08	3.7 (2.8-4.7)	12.9 (9.1-18.2)

Table 3. Bait acceptability and mortality in *T. indica* and *M. hurrianæ* given 'choice' between plain and warfarin-treated bait.

Concentration of poison (per cent)	Duration of test (days)	Mean daily bait intake (g/100 g body wt) Mean \pm S.E.		Significance of student's 't' between 1 and 2	Mortality	Days to death Mean (range)
		Poison (1)	Plain (2)			

<i>Tatera indica</i>						
0.025	14 (2)	1.95 \pm 0.35	4.21 \pm 0.41	0.001	6/12	11.3 (5-19)
0.0125	14 (2)	2.48 \pm 0.45	4.20 \pm 0.59	0.05-0.02	5/12	9.4 (4-14)
<i>Meriones hurrianæ</i>						
0.025	14 (2)	5.46 \pm 0.98	7.16 \pm 1.07	0.30-0.20	11/12	9.7 (5-15)
0.0125	14 (2)	4.07 \pm 0.76	6.94 \pm 0.81	0.02-0.01	8/12	6.1 (4-16)

(Figures in parenthesis indicate the number of days for which bait consumption data were analysed.)

4. Discussion

Our data on toxicity of warfarin against *T. indica* are fairly comparable with that of Greaves and Rehman (1977) in as much as that complete kill was achieved in 14 days feeding on 0.025% warfarin. Comparing the susceptibility of warfarin to gerbils with that of other species it is revealed that they are less susceptible than *R. norvegicus* (Bentley and Larthe 1959; Brooks and Bowerman 1974) and *Bandicota bengalensis* (Deoras 1967; Greaves and Rehman 1977; Sridhara 1979; Brooks *et al* 1980).

The two gerbils under study are also less susceptible than *Arvicanthis niloticus* where 6 days feeding on 0.025% warfarin produced 100% kill (Gill and Redfern 1977). Mukthabai and Krishnakumari (1976) reported 100% kill in *R. rattus* in 7 days, in the same period Mathur and Prakash (1981a) achieved 92% kill, whereas Krishnamurthy *et al* (1968) and Chaturvedi *et al* (1975) observed 100% kill in 13 days. Similar results are also obtained with *Rattus argentiventer* where 10-12 days feeding is required to kill all experimental animals (Buckle *et al* 1980) and *Mastomys natalensis* giving complete kill in 13 days (Gill and Redfern 1979). However, the northern palm squirrel, *Funambulus pennanti*, was found fairly less susceptible to warfarin as compared to the two gerbils where even 14 days feeding could not kill more than 58% squirrels (Mathur and Prakash 1980). *Mus musculus* also required 28 days of feeding on 0.025% warfarin-treated bait for

complete kill (Rowe and Redfern 1964). Significant difference was not observed in the mortality among the two gerbils when the two concentrations, 0.0125% and 0.025% of warfarin were used and hence the former is recommended for the control of *T. indica* and *M. hurrianae*.

Taking the upper 95% confidence limits of LFP_{95} (precluding 0.0125% concentration) the data suggest that feeding on 0.025% warfarin for 18 and 19 days would be suitable to test resistance to warfarin among *T. indica* and *M. hurrianae* respectively. This period is quite comparable with that for *T. indica* (21 days) reported by Greaves and Rehman (1977), *R. argentiventer* (18 days, Buckle *et al* 1980) and cotton rat, *Sigmodon hispidus* (20 days, Gill and Redfern 1980). However, *B. bengalensis* (8 days; Brooks *et al* 1980) and *R. norvegicus* (7 days; Brooks and Bowerman 1974) are more prone to develop resistance to this poison than the gerbils. Greaves and Rehman (1977) also reported that *Tatera* has the potential to develop a significant degree of resistance to anticoagulants than *R. rattus* which requires 28 days feeding on 0.025% warfarin as a suitable test for resistance. It is evident that warfarin provides good results against a number of species but it requires longer period of feeding than brodifacoum and chlorophacinone (Mathur and Prakash 1981b, 1982).

The widespread use of such poison as the sole mean to control gerbils and other rodent pests for a considerable period can eventually lead to pockets of warfarin-resistant animals which is a serious problem in most of Europe and United States of America. It is, therefore, recommended that intermittently other poisons which kill rodents in shorter time should also be used.

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References

- Barnett S A and Prakash 1975 *Rodents of economic importance in India* (New Delhi and London: Arnold-Heinemann) pp. 1-175
- Bentley E W and Larthe Y 1959 The comparative rodenticidal efficiency of five anticoagulants; *J. Hyg. Camb.* **57** 135-149
- Brooks J E and Bowerman A M 1974 An analysis of susceptibilities of several populations of *Rattus norvegicus* to warfarin; *J. Hyg. Camb.* **73** 401-407
- Brooks J E, Htun P T and Naing H 1980 The susceptibility of *Bandicota bengalensis* from Rangoon, Burma to several anticoagulant rodenticides; *J. Hyg. Camb.* **84** 127-135
- Buckle A P, Rowe F P and Yong Y C 1980 Laboratory evaluation of 0.025% warfarin against *Rattus argentiventer*; *Tropical Pest Management* **26** 162-166
- Chaturvedi G C, Madsen C R and Thakore K M 1975 Studies on efficacy of different anticoagulant rodenticides on *Rattus rattus* (House rat); *Proc. All India Rodent Seminar, Ahmedabad* pp. 139-141
- Deoras P J 1967 Tolerance status of some rats to an anticoagulant rat poison; *Curr. Sci.* **36** 207-208
- Finney D J 1971 *Probit analysis* 3rd edn. (Cambridge University Press)
- Gill J E and Redfern R 1977 Some laboratory tests of five rodenticides for the control of *Arvicanthus niloticus*; *PANS* **23** 33-37

- Gill J E and Redfern R 1979 Laboratory tests of seven rodenticides for the control of *Mastomys natalensis*; *J. Hyg. Camb.* **83** 345-352
- Gill J E and Redfern R 1980 Laboratory trials of seven rodenticides for use against the cotton rat (*Sigmodon hispidus*); *J. Hyg. Camb.* **85** 443-450
- Greaves J H and Rehman A B 1977 The susceptibility of *Tatera indica*, *Nesokia indica* and *Bandicota bengalensis* to three anticoagulant rodenticides; *J. Hyg. Camb.* **78** 75-84
- Krishnamurthy K, Uniyal V and Pingale S V 1968 Studies on rodents and their control. IV. Susceptibility of *Rattus rattus* to warfarin; *Bull. Grain. Technol.* **6** 133-137
- Mathur R P and Prakash I 1980 Laboratory evaluation of anticoagulant treated baits for the control of the Northern palm squirrel, *Funambulus pennanti* Wroughton; *J. Hyg. Camb.* **85** 421-426
- Mathur R P and Prakash I 1981a Comparative efficacy of three anticoagulant rodenticides on desert rodents; *Prot. Ecol.* **3** 331-335
- Mathur R P and Prakash I 1981b Evaluation of brodifacoum against *T. indica*, *M. hurrianae* and *R. rattus*; *J. Hyg. Camb.* **87** 179-184
- Mathur R P and Prakash I 1982 Laboratory evaluation of chlorophacinone against *Tatera indica* and *Meriones hurrianae*; *Tropical Pest Management* **28** 291-295
- Mukthabai K and Krishnakumari M K 1976 Responses of *Rattus* species to anticoagulant poisoning; *Comp. Physiol. Ecol.* **1** 129-135
- Prakash I and Jain A P 1971 Bait shyness of the two gerbils *Tatera indica indica* Hardwicke and *Meriones hurrianae* Jerdon; *Ann. Appl. Biol.* **69** 169-172
- Rowe F P and Redfern R 1964 The toxicity of 0.025% warfarin to wild house mice (*Mus musculus* L.); *J. Hyg. Camb.* **62** 389-393
- Sridhara S 1979 Rodenticidal action of poisons on two rodent pests in India; *Pest Control* **47** 30-31
- World Health Organisation 1976 *Instructions for determining the susceptibility or resistance of rodents to anticoagulant rodenticides* (WHO Technical Report Series No. 443)

Effects of handling on oxygen consumption and random activity in the freshwater mullet *Rhinomugil corsula* (Hamilton)

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Abstract. Handling caused excitement which resulted in lower random activity associated with higher rate of oxygen consumption. The routine and standard oxygen consumption rates were increased by 260 and 238%, and 291 and 277% at 30° and 35° C respectively. The temperature effect (30–35° C) did not cause a marked difference ($P < 0.05$) in the rate of oxygen consumption and random activity in *R. corsula*.

Keywords. Handling ; oxygen consumption ; random activity ; respirometer ; *Rhinomugil corsula*.

1. Introduction

Although the rate of oxygen consumption in relation to several factors has been extensively studied in fishes (Spoor 1946 ; Beamish 1964 ; Kutty 1968, 1972 ; Kutty and Peer Mohamed 1975 ; Peer Mohamed and Kutty 1981 ; Peer Mohamed 1981), information on the impact of handling is inadequate (Kutty 1968, 1972 ; Brett 1964 ; Smit 1965). In majority of the studies, the experimental fish was kept in the respirometer for some time in order to recover from the effects of handling, if any, because handling causes excitement and/or random activity to increase (Fry 1967). It has also been reported that the respiratory quotient (RQ) of goldfish and rainbow trout (Kutty 1968) and *Tilapia mossambica* (Kutty 1972) is frequently over unity during periods of excitement. Wedemeyer (1972) found that coho salmon and steelhead trout required 24 hr for normalization of several blood chemistry imbalances after handling. Since it is not known how handling would influence oxygen requirement in fishes, there is need for such information especially on selected cultivable fishes. The present investigation provides an insight on the effects of handling on oxygen consumption and random (spontaneous) activity in the freshwater mullet, *Rhinomugil corsula* (Hamilton). The observations were made at 30° and 35° C ; the high temperatures were chosen because of its relevance to local conditions and because the mullet is exposed to such high temperatures during a good portion of the year.

2. Material and methods

R. corsula, collected from Vaigai Reservoir in South India, ranged in total length from 17.0 to 17.4 cm (mean 17.3 cm; $N = 9$) and in weight from 40.5 to 43.5 g (mean 42.1 g) were used. Fish were acclimated and the observations were made at 30° and 35° C. Two series of experiments were carried out by using a modification of Fry's respirometer (Kutty *et al* 1971) at high ambient oxygen (air saturation); (i) control fish (the fish was left in the respirometer overnight after handling and before experiment and (ii) 'handled' fish (immediately after netting and introducing into the respirometer). The experimental procedure followed was as described in Kutty and Peer Mohamed (1975). Dissolved oxygen in the water samples (50 ml), collected just before and after the closure of the respirometer, was measured by using unmodified Winkler technique (APHA 1955). The random activity was counted by the difference between the initial and final figure of the electronic counter, noted after each sampling. Data obtained on the rate of oxygen consumption and random activity were analysed for fitting regression lines in semilogarithmic grid by least square technique.

3. Results

Regression equations of oxygen consumption (ml/kg/hr) against random activity (counts/hr) in *R. corsula* at 30° and 35° C are given in table 1. Mean values of routine and standard oxygen consumption (extrapolated values to zero activity) and random activity are also included in table 2. The high and low rates of oxygen consumption were estimated to be 98 and 115 ml/kg/hr (30° C) and 105 and 128 ml/kg/hr (35° C) in control fish (series i); 459 and 350 ml/kg/hr at 30° C and 420 and 360 ml/kg/hr at 35° C in *R. corsula* immediately after handling (series ii). The random activity of the fish in series (ii) was low (0-9 counts/hr) and high (10-31 counts/hr) in series (i).

4. Discussion

In the results presented (table 1), a positive correlation between oxygen consumption and random activity in *R. corsula* is noted in both the series (i and ii), which coincides with the observations made earlier in the same species by Kutty and

Table 1. Regression equations ($\log Y = a + bX$) of oxygen consumption (ml/kg/hr) (Y) against random activity (counts/hr) (X) in *Rhinomugil corsula*.

Series (i)—Control fish

$$\log Y = 1.95704 + 0.00342 X \text{ (30° C)}$$

$$\log Y = 1.98213 + 0.00414 X \text{ (35° C)}$$

Series (ii)—'Handled' fish

$$\log Y = 2.54873 + 0.01161 X \text{ (30° C)}$$

$$\log Y = 2.55895 + 0.00713 X \text{ (35° C)}$$

Table 2. Routine and standard oxygen consumption, and random activity in *Rhinomugil corsula*.

	Series	30° C		35° C	
		Routine (mean \pm S.E.)	Standard	Routine (mean \pm S.E.)	Standard
Oxygen consumption (ml/kg/hr)	i	105.7 (7) \pm 2.6	90.6	114.7 (7) \pm 3.1	96
Oxygen consumption (ml/kg/hr)	ii	382.1 (12) \pm 8.3	354	387.5 (12) \pm 6.0	362
Random activity (counts/hr)	i	19.3 (7) \pm 2.7	...	18.4 (7) \pm 2.7	...
Random activity (counts/hr)	ii	2.8 (12) \pm 0.7	...	4.0 (12) \pm 1.0	...

In the case of routine values mean \pm S.E. is indicated. Values in parenthesis denote the number of determinations.

Series i and ii denote control and 'handled' fish respectively (see text).

Peer Mohamed (1975). But, the present data differ from those of Kutty *et al* (1971) in that they observed much higher levels of random activity for *R. corsula* and also a break in activity-oxygen consumption relationship. It is possible that the discontinuity in the relation of oxygen consumption and activity is not evident in the present data because of the narrowness of the range of activity (Kutty 1968 ; Kutty and Peer Mohamed 1975).

The routine and standard oxygen consumption of the control fish (series i) are almost the same as observed earlier (Kutty and Peer Mohamed 1975). It is evident from the results (table 2) that, on comparison of series (ii) with series (i), an upward shift in the rate of oxygen consumption was observed. The routine oxygen consumption rate was shifted by 260 and 238%, and standard rate by 291 and 277%, at 30° and 35° C respectively. The present results thereby suggest that the fish were excited due to handling which resulted in lower random activity associated with high rate of oxygen consumption, that is, the less active the fish, the proportionately higher its energy cost. Fishes could, however, respire up to a level as high as the active metabolic rate due to excitement (Fry 1967). It is likely that the elevated oxygen consumption during lower random activity in the 'handled' fish is accomplished in part by an increase in the transfer factor of the gills (Randall *et al* 1967), that is, the effective exchange area of the gills is increased which results in an increase in the osmotic movement of water. In freshwater, the water moves down the osmotic gradient into the animal.

At both the test temperatures, the rate of oxygen consumption and random activity values are in close proximity and the test of significance showed that the values at 30° and 35° C are not significantly different ($P < 0.05$), suggesting that

and other ovarian components. The present study was, therefore, undertaken to investigate the effects of wall of preovulatory follicle on the ovary of growing chicks till the onset of lay.

2. Material and methods

Three-week old chicks were purchased from the local hatchery and were kept in the laboratory under continuous light and provided with feed and water *ad libitum*. The chicks were allowed to grow till they were 11 weeks old. Then they were divided into three groups of 9 chicks each, keeping the average body weight of a chick similar in all the groups. On alternate days intramuscular injections of 0.5 ml doses of lipid and aqueous extract of wall of largest follicle were given to each chick of the first and second group respectively. The chicks of the third group were injected with saline which served as control. Three chicks from each group were sacrificed at 17th, 23rd and 29th weeks to study the ovarian changes. Killing of chicks before 17th week was avoided as our preliminary studies had shown no visible effect on the ovary up to this time. After counting the number of follicles from the ovarian surface they were fixed in Bouin's fluid and calcium-formaldehyde and subjected to routine histological and histochemical techniques for localization of lipids (Pearse 1968).

For preparation of follicular extracts the follicles measuring 3.8–4.0 cm diameter were separated from the ovary of laying hens. The follicular walls which included both the thecal and granulosa layers were obtained after removal of yolk as described by Huang and Nalbandov (1979). The follicular walls from 10 follicles were then homogenized in saline and after homogenization the material was centrifuged for 15 min at 5000 rpm. The supernatant, thus obtained, was diluted to 50 ml and was kept at 5°C during testing procedures. Similarly the lipid extract of the follicular walls from largest follicles was obtained by extracting the material with chloroform and methanol (2:1 v/v). The lipid extract was dried and was suspended in saline, with slight heating and stirring.

3. Results

Chicks administered with lipoidal extract and killed at 17th, 23rd and 29th week continue to show higher body, ovarian and oviducal weights as compared to control and aqueous extract injected birds (table 1).

At 17th week, no conspicuous differences were observed in the ovarian surface morphology of the lipid and aqueous extract injected hens. But a study of follicular populations from the serial sections of the ovary revealed that the ovaries of chicks, injected with lipid extract, contained some follicles having dimensions more than 500 μm . The other growing follicles like those of control and water extract injected measure less than 400 μm . The number of follicles at different stages of growth (as given in table 1) is relatively more in lipid extract than those of control and aqueous extract injected birds. Follicular atresia affecting mainly the follicles ranging in size from 200–400 μm was common in the ovaries of all the three groups of hens; no significant differences could be observed among them. The interstitial gland cells existed in irregularly distributed patches (figure 1).

Table 1. Mean body, ovary and oviductal weights and number of follicles in 17, 23 and 29 weeks old chicks.

Age of chicks	17 weeks old				23 weeks old				29 weeks old			
	Pure saline	Lipid extract	Water extract		Pure saline	Lipid extract	Water extract		Pure saline	Lipid extract	Water extract	
Weight gain (g)	500	620	500		785	1070	785		860	1080	940	
Ovary weight (mg)	38	51	17		883	1578	455		20600	20300	18600	
Oviductal weight (g)	0.016	0.026	0.017		11.85	42.88	23.60		42.56	47.6	35.6	
Number of follicles (counted from the surface of the ovary):												
(a) Normal follicles		12 (all white)	9 (5 yellow and 4 white)	3 (all large white)		13 (6 yellow 7 white)	15 (8 yellow 7 large white)	9 (5 yellow 4 large white)	
(b) Atretic follicles (yellow)		Nil	3	Nil		Nil	3	7	
Mean number of follicles (in serial sections):												
(i) 75-160 μ m	10	14	6		20	48	2		17	18	15	
(ii) 200-270 μ m	10	17	4		7	30	9		8	6	4	
(iii) 300-400 μ m	3	12	3		18	14	4		11	7	8	
(iv) 500-1300 μ m	...	3	...		6	7	2		3	3	3	

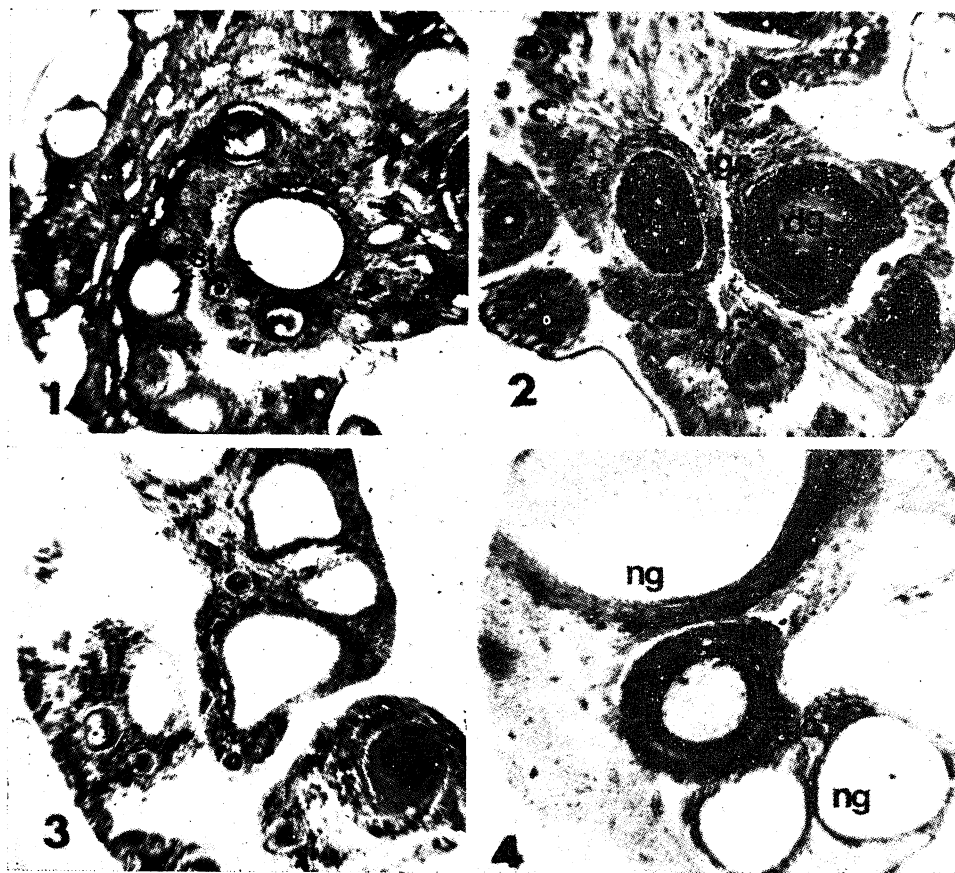
Ovaries of hens at 23rd week possessed follicles of larger size, some of which could be counted easily from the ovarian surface. Marked differences were seen in the ovarian surface morphology of hens which were continuously administered with lipid extract because they started laying at 22nd week. Ovaries of these birds were having normal hierarchical follicles whereas those of control and aqueous extract injected chicks contained only large white follicles, their number was also relatively less in the latter (table 1). Atresia of follicles is increased considerably at 23rd week as compared to the ovaries of 17-week old chicks but it was more in ovaries treated with the water extract and affected the previtellogenic follicles (figures 2, 3). Vitellogenic atretic follicles were also observed in the lipid extract administered hens. The ovarian stroma of the 23-week old chick was found to contain abundant interstitial gland tissue, but their amount was relatively more in aqueous extract injected birds (figures 3, 4). In the latter case, the interstitial gland tissue contained more sudanophilic lipids as compared to those of control and lipoidal extract administered chicks.

At 29th week, the ovaries of control and lipid extract injected birds showed the normal features of those of laying hens, since the control birds also started laying at the 25th week. But the aqueous extract injected birds started laying at the end of the 29th week. The ovaries of these birds contained more number of medium-sized vitellogenic atretic follicles as judged from the shrinkage and prominence of the stigmal site. The ovarian stroma at this stage appeared relatively loose in all the three treatment groups. The interstitial gland cells were abundant and continued to show more lipids in aqueous extract injected hens.

4. Discussion

The present observations have shown that the lipid and aqueous extracts of walls of larger yellow follicles have antagonistic effects on the ovarian functions in the growing chicks. The lipoidal extract initiates the follicular growth from the pool and enhances the rate of growth of follicles at all stages leading to their early maturity. However, the aqueous extract appears to have the reverse effect. The enhanced rate of growth of follicles, thus, indicates the presence of some lipid-like growth-promoting substance elaborated by the larger follicles. *In vivo* and *in vitro* studies have shown that the largest follicle secretes progesterone (Furr *et al* 1973 ; Shodono *et al* 1975 ; Shahabi *et al* 1975 ; Huang *et al* 1979) and prostaglandins (Hammond *et al* 1980) shortly before ovulation. Thus the presence of these two substances in the lipid homogenate of the follicular walls is expected but the possibility of the existence of any other lipoidal substance cannot be excluded. Prostaglandins do not appear to influence ovarian steroidogenesis in hen (Hertelendy and Hammond 1980), but their role in initiating and promoting follicular growth is not known. The involvement of progesterone in promoting follicular growth cannot be overlooked since it is known to play a key role in endocrine control of the hypothalamo-pituitary-ovarian axis.

The effect of growth-promoting substance expected to be present in the lipoidal extract becomes more marked after 17th week. Thus it appears that pituitary-ovarian axis after 17th week probably becomes more responsive to the growth-promoting factor contained in lipid extract of the walls of the largest follicle,



Figures 1-4. 1. Section of 17-week old chick administered with aqueous extract showing small growing follicle(s) and patches of interstitial gland cells in stroma. Sudan black B \times 50. 2. Section of ovary of 23-week old chick administered with aqueous extract showing degenerating follicles (*dg*) and abundant lipid-rich interstitial gland cells (*igc*). Sudan black B \times 50. 3. Section of the ovary of 23-week old chick injected with aqueous extract showing abundant interstitial gland cells (*igc*) in the stroma. Sudan black B \times 50. 4. Section of ovary of 23-week old chick injected with lipoidal extract showing normal growing follicles (*ng*) and stroma with lesser interstitial gland cells (*igc*). Sudan black B \times 50.

In contrast to the lipid extract, the aqueous extract of the follicular wall inhibits the follicular growth and simultaneously enhances follicular atresia. A water-soluble factor was extracted from the largest preovulatory and postovulatory follicles which could induce premature oviposition (Tanaka and Nakada 1975) but no mention is made until now regarding its effect on the ovary itself. Preliminary studies on the estimation of soluble proteins have indicated that the amount of proteins abruptly increases in the follicles of 3.8–4.0 cm diameter (unpublished observations). Possibly, there may be same protein in aqueous extract which exerts inhibitory influence on the follicular growth and stimulation of follicular atresia but this suggestion needs to be extended and confirmed.

Our observations on the ovary of growing chicks after treatment with lipoidal and aqueous extracts have clearly shown that the larger yellow follicles in the laying hen elaborate two different kinds of substances, one stimulates and the second possibly inhibits the follicular growth. But the exact mechanisms of action of these two factors in maintaining the normal and regular pattern of laying remains to be determined.

References

- Etches R J and Cunningham F J 1976 The interrelationship between progesterone and luteinizing hormone during the ovulatory cycle of the hen (*Gallus domesticus*); *J. Endocrinol.* **71** 51–58
- Furr B J A, Bonney R C, England R J and Cunningham F J 1973 Luteinizing hormone and progesterone in peripheral blood during ovulatory cycle of the hen *Gallus domesticus*; *J. Endocrinol.* **57** 159–169
- Graber J W and Nalbandov A V 1976 Peripheral estrogen levels during the laying cycle of the hen (*Gallus domesticus*); *Biol. Reprod.* **14** 109–114
- Hammond R W, Koelkebeck K W, Scanes C G, Biellier H V and Hertelendy F 1980 Prostaglandins and steroid hormones in the plasma and ovarian follicles during the ovulatory cycle in domestic hen (*Gallus domesticus*); *Gen. Comp. Endocrinol.* **42** 195–202
- Hertelendy F, Yeh M and Biellier H V 1974 Induction of oviposition in the domestic hen by prostaglandins; *Gen. Comp. Endocrinol.* **22** 529–531
- Hertelendy F and Hammond R W 1980 PGs do not affect steroidogenesis and are not produced in response to oLH in chicken granulosa cells; *Biol. Reprod.* **23** 918–923
- Huang E S and Nalbandov A V 1979 Steroidogenesis of chicken granulosa and theca cells; *In vitro* incubation system; *Biol. Reprod.* **20** 442–453
- Huang E S, Kao K J and Nalbandov A V 1979 Synthesis of sex steroids by cellular components of chicken follicles; *Biol. Reprod.* **20** 454–461
- Haynes N B, Cooper K J and Kay M J 1973 Plasma progesterone in the hen in relation to the ovulatory cycle; *Br. Poult. Sci.* **14** 349–357
- Kumagai S and Homma K 1974 High estrogen production of the medium-sized follicles during follicular growth and ovulation in laying quail; *Endocrinol. Jpn.* **21** 349–354
- Lance V and Callard I P 1979 Hormonal control of ovarian steroidogenesis in non-mammalian vertebrates. In *The vertebrate ovary*, (eds.) R E Jones (New York and London: Plenum Press) Chap. 10, pp. 361–407
- Pearse A G E 1968 *Histochemistry—Theoretical and applied* (London: J & A Churchill Ltd.)
- Shahabi N A, Norton H W and Nalbandov A V 1975 Steroid levels in follicles and plasma of hens during the ovulatory cycle; *Endocrinology* **96** 962–968
- Shodono M, Nakamura T, Tanabe Y and Wakabayashi K 1975 Simultaneous determinations of oestradiol-17 β , progesterone and luteinizing hormone in the plasma during the ovulatory cycle of the hen, *Acta Endocrinol.* **78** 565–573
- Tanaka K and Nakada T 1974 Participation of the ovarian follicle in control of time of oviposition in the domestic fowl; *Poult. Sci.* **53** 2120–2125

Biochemical studies on the haemolymph and heart muscle of normal and insecticide treated cockroach *Periplaneta americana* L.

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Abstract. In this paper quantitative estimations of free proteins, total carbohydrates/glycogen and fatty acids from cockroach haemolymph and heart muscle are reported from normal and insecticide treated insects. High content of protein, carbohydrate/glycogen and fatty acids are found in the haemolymph and heart muscle of nymphal insects. Higher amount of carbohydrates/glycogen are found in adult males, while more protein and fatty acids were found in the females. After insecticide treatment, no sex variation was found in the percent depletion of metabolites. The difference in the depletion rates between nymph and adult was also insignificant. High percent depletion of the macro-molecules was found with insecticides which are found more toxic in bioassay studies. A correlation has been made between the rate of depletion and insecticidal poisoning.

Keywords. Insecticide ; haemolymph ; heart muscle ; carbohydrates ; glycogen proteins ; fatty acids.

1. Introduction

It is generally known that, insecticides interfere with the physiology of insect nervous system, particularly with the nerve conduction mechanism. However, with the lack of sufficient data the ultimate causes of death in insects are usually difficult to prove.

Information on carbohydrate, protein and lipid levels from various tissues of insect is scanty as compared to vertebrates. Only in recent years, insect blood has been studied for both normal and after treatment with some insecticides, especially the chlorinated hydrocarbons (Corrigan and Kearns 1963 ; Hawkins and Sternburg 1964). These studies are limited since the observations were only made on isolated fractions such as amino acids, free sugars or total lipids.

Despite years of research (Jones 1974 ; Florkin and Jeuniaux 1974), limited information is available on the changes in heart-beat and much less on heart muscle due to insecticide action.

The present studies were undertaken to find out the effect of certain insecticides on basic metabolite content of cockroach haemolymph and heart muscle and its significance in the poisoning of the insects. A description of the quantitative variations in the total carbohydrates, proteins and fatty acids from the haemolymph and heart muscle of the last instar nymph and adult cockroach, *Periplaneta americana* L. are given. The effects of a plant extract 'Morindin' reported to be toxic to insects (Surender Reddy *et al* 1978) is also mentioned.

2. Material and methods

The test insects *Periplaneta* were collected and developed at room temperature in glass cages with a wire mesh lid at the top. A layer of sawdust was laid on the floor of the cage for the deposition of oothecae. Once in a week the oothecae deposited were separated to another cage for hatching. The insects were fed on glucose biscuits mixed with yeast powder and potato peels. The following insecticides were used.

Fenitrothion : O,O-dimethyl-o-(3-methyl-4-nitrophenyl)-thionophosphate (Baeyer India Limited, Bombay) ; Carbofuran : 2,3-dihydro-2,2-dimethyl-7-Benzofuranyl methyl carbamate (Rallis India Limited, Bangalore) ; Ekalux : 25% (w/w) Quinolphos (O,o-diethyl-o (quinoxaliny)-(2) Thionophosphate) and 75% (w/w) stabilizers, emulsifiers and other adjuvants (Sandoz India Limited, Bombay) ; Morindin : The glycoside morindin 6-primeveroside of morindone ($C_{26}H_{28}O_{14}$, 1,5,6-trihydroxy 2-methyl anthraquinone) has been extracted and purified from the root bark of *Morinda tinctoria* var. *tomentosa* Hook. as described by Rao and Reddy (1977) ; Nicotine : Manufactured by E Merck, Darmstadt, Germany. The insecticides were dissolved in ethyl alcohol and insects were treated intraperitoneally (Menusan 1948) with the help of an Agla micrometer syringe. Insects to be treated were weighed individually and the dosage was calculated per gram (5 μ l/g) of the body weight. Last instar nymphs of developing wing base were selected to maintain uniformity of age. Adults belong to the age group of 1-4 days after molt. After determining the lethal dosages, one producing 50% kill (LC_{50}) was selected for the present experiments and the insects were taken for biochemical estimations 4-6 hours after treatment. This particular time lag was chosen because the initial symptoms of poisoning such as hyperactivity and convulsions were complete in less than 4 hr, thus the insects were with complete knock down effect. Besides the normal insects, control insects were taken 4-6 hr, after treatment with ethyl alcohol (5 μ l/g).

Haemolymph was collected according to the method described by Sternburg and Corrigan (1959) and the haemocytes were not allowed to sediment. For cardiac muscle, insect heart was fully exposed with specially made needles and carefully separated from the alary muscles throughout the length, then it was gradually lifted on to a cover slip to weigh it gently before transferring into the test tube. Care was taken to eliminate all foreign tissue associated with heart, including the alary muscles and fat content.

To estimate the total carbohydrates from haemolymph and glycogen from heart muscle, the modified anthrone method of Klicpera *et al* (1957) was adopted. For proteins the procedure of Lowery *et al* (1951) and for total/esterified fatty acids, the methods of Stern and Shapiro (1953) were followed.

3. Results

The values of total carbohydrates/glycogen, total proteins and fatty acids recorded from the normal nymphs and adult cockroaches of both sexes are mentioned in tables 1 and 2. It may be seen that, the three constituents of haemolymph of adult insects are relatively lower than those of nymphs. Haemolymph from nymphal cockroach shows about 15–20% more of carbohydrates, proteins and fatty acids. Among the adults, males show 15% more carbohydrates than females, while females possess 18% more proteins and 27% more fatty acids than males. In the heart muscle also it is observed that, nymphs of both the sexes possess relatively higher values of glycogen (25–30%), proteins (13–20%), and fatty acids (17–23%) as compared with adults. In adult cockroaches, glycogen content of heart is about 20% more in males while proteins and fatty acids are about 18% and 20% higher in females. In the control insects treated with ethyl alcohol slight decrease in the total content was seen as compared to normal.

In haemolymph, the percent depletion caused due to fenitrothion treatment in relation to control values were 33–38% in proteins, 22–24% carbohydrates and 20–39% fatty acids. With carbofuran, 52–60% proteins, 23–40% carbohydrates and 33–42% fatty acids, which is considered to be highly significant. After ekalux treatment, 20–30% of proteins, 14–42% of carbohydrates and 11–17% of fatty acids were found to be depleted. After morindin application, 15–26% proteins, 11–28% carbohydrates and 8–28% fatty acids were found to be depleted. Similarly, nicotine caused 17–20%, 10–15% and 5–14% depletion in proteins, carbohydrates and fatty acids respectively (table 1). The concentration of insecticides employed are given in the table.

In heart muscle, the percent depletion observed after fenitrothion treatment in the nymphal and adult cockroaches of both sexes were glycogen 24–33%, proteins 18–21% and fatty acids 17–38%. After carbofuran treatment, glycogen 29–35%, proteins 24–47% and fatty acids 24–40%. After ekalux, glycogen 20–26%, proteins 11–17% and fatty acids 10–18% were found to be depleted. With nicotine treatment, glycogen 10–16%, proteins 2–6% and fatty acids 4–8%, while with morindin, glycogen 12–18%, proteins 4–10% and fatty acids 5–16% were depleted (table 2).

4. Discussion

Quantitative studies on the total proteins, carbohydrates and fatty acids from the haemolymph of normal cockroach give a general indication of higher content in the nymphs than adults. It is suggested that the initial high values of protein and aminoacids in the young ones and the rapid fall during adult stage was associated with the completion of maturation processes involving protein synthesis during the first few days after the final moult (Nowosielski and Patton 1965). Relatively lower content of fatty acids and carbohydrates in adults, as found in the present study, can be attributed to the higher rate of metabolism during metamorphosis (Weis-Fogh 1952; Guthrie and Tindall 1968). High amounts of total proteins and fatty acids found in female cockroaches are in agreement with the findings of Nath *et al* (1958) and Anderson (1964).

As in case of haemolymph, the normal values of glycogen, total proteins and fatty acid contents from the cardiac muscle of *P. americana* (alary muscles

Table 1. Effect of different insecticides on carbohydrates, proteins and fatty acids of cockroach haemolymph.

Content	Normal std. error	Control	Fenitro- thion	Carbo- furan	Ekalux	Nicotine	Morindin
			*N 0.2 A 1.0	0.05 0.06	0.2 0.4	0.25 0.75	0.15 0.25
Total carbohydrates							
N	1070±39	1020±50	780±38	700±38	840±32	910±21	900±23
A	910±47	850±70	620±62	650±62	570±78	720±40	610±50
N	920±32	870±25	680±29	630±36	740±16	760±29	730±26
A	770±65	750±40	510±49	440±18	430±19	700±25	537±24
Proteins							
N	1000±39	900±50	600±26	360±19	720±26	740±24	760±17
A	840±53	800±60	500±60	320±77	560±77	640±112	600±77
N	1200±33	1100±30	700±29	520±27	820±30	880±16	900±29
A	1020±98	980±60	600±50	440±20	700±77	780±101	720±76
Fatty acids							
N	300±19	280±25	190±17	160±26	230±27	240±26	200±26
A	240±34	210±30	160±20	140±22	185±18	195±19	180±21
N	400±23	380±15	280±24	230±28	320±21	360±18	340±20
A	330±32	300±25	240±19	200±21	260±20	285±28	275±44

Values represent the average of 20 individuals, expressed in mg per 100 ml haemolymph.
N : nymph ; A adult ; * concentration of insecticides expressed in µg/insect.

Table 2. Effect of different insecticides on glycogen, proteins and fatty acids of cockroach heart muscle.

Content	Normal std. error	Control	Fenitro- thion	Carbo- furan	Ekalux	Nicotine	Morindin
			*N 0.2 A 1.0	0.05 0.06	0.2 0.4	0.25 0.75	0.15 0.25
Glycogen							
N	2580±104	2500±95	1720±89	1630±92	1840±93	2100±78	2050±96
A	1918±80	1900±80	1470±92	1350±101	1520±98	1700±98	1658±101
N	2270±128	2220±100	1640±82	1560±86	1720±89	1917±89	1888±89
A	1520±87	1470±100	1060±78	980±72	1140±51	1305±49	1250±82
Proteins							
N	2826±97	2760±96	2268±101	2089±98	2448±78	2695±79	2640±106
A	2250±110	2200±100	1726±89	1608±90	1833±104	2149±48	2043±89
N	3168±118	3090±115	2452±87	2286±119	2620±87	2896±112	2768±122
A	2742±120	2675±110	2160±88	1920±92	2280±86	2509±88	2390±124
Fatty acids							
N	680±78	610±72	505±42	463±46	562±32	584±40	575±38
A	520±50	480±65	388±36	320±39	409±29	440±20	428±30
N	780±70	750±60	564±72	502±42	610±84	706±88	628±92
A	648±82	600±62	450±42	390±40	504±42	560±46	520±49

Values represent the average of 20 individuals, expressed in µg per 100 mg w.w. of muscle.
N : nymph ; A : adult ; * concentration of insecticides expressed in µg/insect.

excluded) also show an increase in nymphs. A similar decrease in the glycogen content of adult locust muscles was observed by Chari (1970). A decrease in the content of metabolites of adult cockroach heart muscle (observed in the present studies) appear to be due to their utilization during metamorphosis from young to adult, as it was emphasized by Rockstein (1964).

It has been observed that sexual variation is higher than the influence of age on the concentration of the basic constituents. Males have high amount of carbohydrate/glycogen while females have higher amount of protein and fatty acids. This is true for the haemolymph as well as for the heart muscle. Such similarity in the metabolite ratios between the insect haemolymph and heart muscle reveals, perhaps, their physiological association. It is well known that the haemolymph, having a number of reserve transport material, constantly circulates between the dorsal tubular heart and body cavity. The heart is a connective tissue, pulsating and pumping the blood which enters it through the ostia and is emptied through the dorsal aorta.

In the insects treated with insecticides, haemolymph proteins were depleted the most, followed by carbohydrates and fatty acids. While in heart muscle, the difference in the percent depletion of three metabolites was however not significant. This is applicable for nymphs and adults of both sexes. More percent depletion was observed with carbofuran followed by fenitrothion > ekalux > morindin > nicotine. In general, the degree of percent depletion found in the three metabolites do not vary much between one another. However, the percent depletion noted in blood proteins is found to be significantly higher.

As in case of vertebrates, binding of insecticides both to cellular components and soluble proteins in insects is suggested by Olson (1973). The small and insignificant difference found in macromolecule content of control insects may be attributed to the dilution of haemolymph after solvent treatment.

Relatively low depletion of proteins, carbohydrates/glycogen and fatty acids found with nicotine is in agreement with its low toxicity in the bio-assay studies (Surender Reddy 1979). This may be attributed to quick metabolism and excretion of nicotine from the insect body. Extensive metabolism of nicotine when fed to grasshoppers or applied topically to house-flies was observed by Self *et al* (1964). With tobacco hornworm it is reported that 90% of oral dose of nicotine was excreted in about 4 hr while 83% of nicotine injected into the body cavity was seen in feces in about 15 min (Self *et al* 1964).

From the present studies it appears that, besides the target tissue like central nervous system, susceptibility of insects to an insecticide will also be necessarily accompanied by biochemical variations in other vital tissues, proportionate to the toxicity of the substance. A similar conclusion was drawn by Mansingh (1965) in his studies with *Blattella germanica*. It is also corroborated by the opinion of other workers (Hollingsworth 1976), that besides acetylcholinesterase there exist other targets in the insecticide poisoning of insects.

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References

- Anderson E 1964 Oocyte differentiation and vitellogenesis in the cockroach *Periplaneta americana*; *Cell. Biol.* **20** 131-155
- Chari N 1970 Metabolic differentiation of functionally different muscles of some insects and other invertebrates. C.Sc. Thesis CSAV Praha
- Cornwell P B 1976 *The cockroach*, Vol. II (London: Assoc. Business Progr. Ltd.) p. 318
- Corrigan J J and Kearns C W 1963 Amino acid metabolism in DDT poisoned American roaches; *J. Insect. Physiol.* **9** 1-12
- Florkin M and Jeuniaux C 1974 Haemolymph composition. In *The Physiology of Insecta* Vol. 5 (ed.) M. Rockstein (New York, London: Academic Press) pp. 256-302
- Guthrie D M and Tindall A R 1968 *The Biology of the cockroach* [London: Edward Arnold (Publishers) Ltd.] pp. 98, 287, 332
- Hawkins W B and Sternburg J 1964 Some chemical characteristics of a DDT induced neuro-active substance from cockroach and cray fish; *J. Econ. Entomol.* **57** 241-247
- Hollingworth R M 1976 The biochemical and physiological basis of selective toxicity. In *Insecticide biochemistry and physiology* (ed.) C F Wilkinson (New York and London: Plenum Press) pp. 431-497
- Jones J C 1974 The circulatory system of insects. In *The Physiology of Insecta*, (ed.) Vol. 3 M. Rockstein (New York, London: Academic Press) p. 121
- Klicpera H, Drahotka Z and Zak R 1957 Notes on the determination of muscle glycogen; *Physiol. Bohemoslov.* **6** 569
- Lowery O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein estimation with folin-ciocalteu reagent; *J. Biol. Chem.* **193** 256-272
- Mansingh A 1965 The effect of malathion on the metabolism of amino acids in the German cockroach *B. germanica*; *J. Insect Physiol.* **11** 1389-1400
- Menusan Jr H, 1948 Comparative toxicity of insecticides administered in various ways to several species of insects; *J. Econ. Entomol.* **41** 302-313
- Nath V, Gupta B L and Lal B 1958 Histochemical and morphological studies of the lipids in oogenesis in *Periplaneta americana*; *Q. J. Microsc. Sci.* **99** 315-322
- Nowosielski J W and Patton R L 1965 Variation in the haemolymph protein, amino acid and lipid levels in adult house cricket, *Acheta domesticus* L. of different ages; *J. Insect Physiol.* **11** 263-270
- Olson W P 1973 Dieldrin transport in the insect—an examination of Gerolts hypothesis; *Pestic. Biochem. Physiol.* **3** 384-392
- Rao P S and Reddy G C V 1977 Isolation and characterization of the glycoside of morindone from the root bark of *Morinda tinctoria* var. *tomentosa*; *Indian J. Chem.* **B15** 497-498
- Rockstein M 1964 Biology of the Insecta. In *The Physiology of Insecta* Vol. 1 (ed.) M. Rockstein (New York, London: Academic Press) pp. 3-8
- Self L S, Guthrie F E and Hodgson E 1964 Metabolism of nicotine by certain insects; *Nature (London)* **204** 300
- Strenberg J and Corrigan J J 1959 Rapid collection of insect blood; *J. Econ. Entomol.* **52** 538-539
- Stern I S and Shapiro B 1953 Calorimetric estimation of esterified differences in insect blood proteins; *Physiol. Zool.* **30** 114-120
- Surender Reddy G, Purushotham Rao A and Rao P S 1978 Preliminary observations of the toxicity of morindin, a glycoside to cockroaches and houseflies; *J. Food Sci. Tech.* **15** 86
- Surender Reddy G 1979 Biochemical and physiological effects of certain insecticides on cockroach, *Periplaneta americana*. Ph.D. Thesis, Kakatiya University, Warangal, A.P., India
- Weis-Fogh T 1952 Lipid metabolism during insect metamorphosis; *Phil. Trans. Roy. Soc. London* **B237** 1

Fecundity of a hillstream minor carp *Puntius chilinoides* (McClelland) from Garhwal Himalaya

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Abstract. One hundred mature specimens of *P. chilinoides* collected from the Badiyar gaad, a tributary of the river Alaknanda were examined for fecundity. The fish weight, ovary weight, and fecundity ranged from 25-115 g, 2.1-14.35 g, and 2135-7974 respectively. The ovary weight was found from 8.4 to 16.34% of the body weight. The relationships between fecundity and total length and weight of fish, fecundity and length, weight and volume of ovary, fish length-ovary weight, and fish weight-ovary weight were found to be of linear form.

Keywords. Fecundity; *Puntius chilinoides*; fish weight; ovary weight.

1. Introduction

Fecundity of a fish may be defined as the number of eggs that are likely to be laid during a spawning period. Studies on the fecundity of fishes are useful for increasing the yield of consumable fish species. However, so far no studies have been made on the fecundity of coldwater fishes of Garhwal Himalaya. Hence it was considered desirable to study the fecundity of *P. chilinoides*, an important food-fish found in the tributaries of the Alaknanda.

2. Materials and methods

Hundred specimens of mature *P. chilinoides* were collected from Badiyar gaad, a tributary of the river Alaknanda of Garhwal Himalaya during March-April 1980 and 1981. The total length and weight of each fish and ovary in fresh condition were noted. The ovary of each fish was dissected out and preserved in 5% formalin solution for 24 hrs. The fecundity of the fish was recorded by gravimetric method (Simpson 1959) and studied in relation to its weight and total length, and length, weight and volume of ovary. These relations have been expressed as follows by applying the method of least square.

(i) The straight line $Y = a + bX$ (ii) $Y = axb$ or in logarithmic form as

$$\log Y = \log a + b \log X$$

3. Observations

3.1. Fecundity and fish length

The relationship between fecundity and total length of fish is shown in table 1. According to mean values the number of ova varied from 2097 for a fish of 130 mm to 7978 in the fish measuring 220 mm, while the minimum fecundity was 2080 in a fish of 135 mm. The largest specimen of 217 mm had a fecundity of 8020. The relationship between fecundity and total length in the logarithmic form can be expressed as :

$$\log F = 3.56 + 1.825 L$$

where F = fecundity in thousands and L = total length in mm. The fecundity-length relationship in *P. chilinoides* can be expressed as :

$$F = -0.15 + 100 L \quad (r = 0.9112).$$

3.2. Fecundity and fish weight

The relationship between fecundity and fish weight is shown in table 2. Egg production ranged from 2115 in a fish of 2.1 g to 8020 in a fish of 14.6 g. The fecundity-body weight relationship in *P. chilinoides* can be expressed as :

$$F = -2150 + 100 WF$$

where WF is the total weight of the fish in g. The relationship between fecundity and body weight in logarithmic form can be expressed as :

$$\log F = 3.16 + 2.227 \log WF \quad (r = 0.8767)$$

3.3. Fecundity and ovary weight

The relationship between ovary weight and fecundity was found to be close and linear in nature. The correlation coefficient, r , is 0.9493, which indicates that

Table 1. Relationship between fish length, ovary weight and fecundity in *P. chilinoides*.

Total length (mm) of fish range	Mean (mm)	No. of fish examined	Ovary weight (g)		Number of eggs	
			Range	Average	Range	Average
125-135	130	2	2.00 - 2.100	2.050	2080-2115	2097
135-145	140	15	2.100 - 4.200	2.733	2122-3035	2543
145-155	150	21	4.00 - 6.500	5.128	3837-5747	4963
155-165	160	14	6.400 - 7.450	6.975	5680-6380	5956
165-175	170	20	6.700-10.600	8.327	6485-7090	6851
175-185	180	17	10.300-12.300	11.108	7081-7750	7398
185-195	190	6	11.900-13.200	12.400	7680-7788	7719
195-205	200	1	13.400	13.400	7820	7820
205-215	210	2	13.800-14.00	13.900	7845-7935	7890
215-225	220	2	14.100-14.600	14.350	7929-8020	7978

Table 2. Relationship between fish weight, fecundity and ovary weight in *P. chilinoides*.

Weight of fish (g) Range	Mean (g)	No. of fish examined	Fecundity		Ovary weight (g)		% of ovary weight in total weight of fish
			Range	Mean	Range	Mean	
20-30	25	5	2080-2186	2135	2.00-2.200	2.100	8.40
30-40	35	24	2285-4950	3618	2.400-5.200	3.714	10.61
40-50	45	18	5050-5992	5652	5.250-7.200	6.363	14.14
50-60	55	22	5921-6990	6661	6.900-10.600	7.468	13.57
60-70	65	11	7020-7392	7201	9.900-11.400	10.622	16.34
70-80	75	15	7420-7788	7602	10.300-13.200	11.793	15.72
80-90	85	1	7820	7820	13.400	13.400	15.76
90-100	95	1	7845	7845	14.00	14.00	14.73
100-110	105	1	7935	7935	13.800	13.800	13.14
110-120	115	2	7929-8020	7974	14.100-14.600	14.350	12.47

the fecundity is more directly related to the weight of the ovary. Egg production ranged from 2115 in an ovary of 2.1 g to 8020 in an ovary of 14.6 g. The fecundity-ovary weight relationship may be expressed as :

$$F = 3350 + 354.1 WO ; \text{ where } WO = \text{weight of ovary}$$

$$\log F = 3.065 + 0.555 (r = 0.9493)$$

3.4. Fecundity and ovary length

The fecundity increased with length of ovaries. This relationship can be expressed as :

$$F = -0.09 + 250 LO$$

$$\log F = 2.09 + 2.794 \log LO ; (r = 0.9629)$$

where LO is the length of ovary.

3.5. Fecundity and ovary volume

Fecundity increased with the volume of ovaries. The data on the volume of ovary and fecundity can be expressed as :

$$F = 3100 + 423 VO ;$$

$$\log F = 3.538 + 0.475 \log VO ; r = 0.9384$$

where VO = the volume of ovary.

3.6. Ovary weight and fish weight

The relationship between the fish weight and ovary weight can be expressed as :

$$WO = -2.8 + 2 FW$$

The same relationship in logarithmic form may be expressed as :

$$\log WO = 0.21 + 2.5 \log WF ; r = 0.9597$$

where WF = weight of fish

3.7. Ovary weight and fish length

The relationship between total length of fish and ovary weight was found to be fairly close and linear in nature, the 'r' being 0.9862 appears to be the highest amongst all relationships. It indicates that fish length is more directly related to ovary weight. The relationship between length and ovary weight may be expressed as :

$$OW = 0.3 + 1.6 FL$$

$$\log OW = -0.854 + 0.202 \log FL; r = 0.9862.$$

where OW = weight of ovary and FL = length of fish.

4. Discussion

Various investigators like Clark (1934), Khan (1945), Smith (1947), Lehman (1953), Alikunhi (1956), Mathur (1964), Saigal (1964), Bhatnagar (1964), Alikunhi *et al* (1965), Rangarajan (1971), Devraj (1973), Varghese (1973, 1976), Chondar (1977), and Joshi (1980), have studied the fecundity of several fish species. The relationships have been found to exist between the length and fecundity of different species of fish. Clark (1934) suggested that the fecundity of a fish increased in proportion to the square of its length. Simpson (1951) concluded that the fecundity of plaice was related to the cube of its length. Relationship between fish length and fecundity has been reported by Sarojini (1957), Pantula (1963), Gupta (1968), Varghese (1973), and Joshi (1980). However, in *P. chilinoides*, the fecundity increases with increase in fish length.

A straight line relationship between the fish weight and fecundity has been reported by several workers including Begenal (1957), Sarojini (1957), and Varghese (1961, 1973). A curvilinear relationship was found in *Coilia ramcarati* (Varghese 1976), but in *P. chilinoides* a straight line relationship has been found between the fish weight and fecundity. In *Salvelinus fontinalis* the fecundity is related more to the weight than the length of fish (Smith 1947). A direct proportional increase in the fecundity with the increase in the fish weight has been noted by Simpson (1951) and Lehman (1953). In *P. chilinoides* also there is an increase in the number of eggs with the increase in the body weight.

This paper shows that the fecundity and fish length relationship ($r = 0.9112$) is more closely related than the fish weight and fecundity ($r = 0.8767$). The linear relationship between the volume of ovary and fecundity indicates an increase in the number of ova produced with the volume of ovaries. Therefore, it appears that the fecundity increases at a smaller rate in respect to the volume of ovary.

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References

- Alikunhi K H 1956 Observations on fecundity, larval development and early growth of *Labeo bata* (Ham.); *Indian J. Fish.* 3 216-229
- Alikunhi K H, Sukumaran K K and Parmeswaran S 1965 Observations on growth, maturity and breeding of induced bred, pond-reared silver carp, *Hypophthalmichthys molitrix* and grass carp, *Ctenopharyngodon idellus* in India during July 1962 to August 1963; *Cent. Inst. Fish. Education Bulletin* No. 2
- Begenal T B 1957 The breeding and fecundity of the long rough dab, *Hippoglossoides platessoides* (Fabr.) and the associated cycle in condition; *J. Mar. Biol. Assoc. U.K.* 36 339-375
- Bhatnagar G K 1964 Observations on the spawning frequency and fecundity of certain Bhakra reservoir fishes; *Indian J. Fish.* 11 485-502
- Chondar S L 1977 Fecundity and its role in racial studies of *Gadusia chapra*; *Proc. Indian Acad. Sci.* B86 245-254
- Clark F N 1934 Maturity of California Sardine (*Sardinella caerulea*) determined by ova diameter measurements; *Fish. Bull. California* pp. 42-49
- Devraj M 1973 Biology of the large snake head *Ophiocephalus marulius* (Ham.) in the Bhawani Sagar water; *Indian J. Fish.* 20 280-309
- Gupta M V 1968 Observations on the fecundity of *Polynemus paradiseus* Linn. from the Hooghly estuarine system; *Proc. Natl. Inst. Sci. India* 34 330-345
- Joshi S N and Khanna S S 1980 Relative fecundity of *Labeo gonius* (Ham.) from Nanaksagar reservoir; *Proc. Indian Acad. Sci. (Anim. Sci.)* 89 493-503
- Khan H 1945 Reproductive powers and breeding habits of some of the fishes of Punjab; *Punjab Fish. Manu.* (Lahore), Appendix 2 pp. 6-11
- Lehman B A 1953 Fecundity of Hudson river shad; *Res. Rep. Fish. Bull. U.S.* pp. 121
- Mathur P K 1964 Maturity and fecundity of *Hilsa ilisha*; *Indian J. Fish.* 11 423-448
- Pantula V R 1963 Studies on the age growth and fecundity and spawning of *Ostiogeteiosus militaris* (Linn.); *J. Com. Int. Explor. Mer.* 28 295-315
- Rangarajan K 1971 Maturity and spawning of the Snapper, *Lutianus kasmira* (Forsk.) from the Andaman sea; *Indian J. Fish.* 18 114-125
- Saigal B N 1964 Studies on the fishery and biology of the commercial cat fishes of the Ganga river system. II. Maturity, spawning and food of *Mystus aor* (Ham.); *Indian J. Fish.* 11 1-44
- Sarojini K K 1957 Biology and fisheries of the grey mullets of Bengal. I. Biology of *Mugil parsia* (Ham.) with notes on its fishing in Bengal; *Indian J. Fish.* 4 160-207
- Simpson A C 1951 Fecundity of plaice; *Fish. Inves. London* 17 1-27
- Simpson A C 1959 Method used for separating and counting the eggs in fecundity studies on the plaice (*Pleuronectes platessa*) and herring (*Clupea herengus*); *Occ. Pap. FAO, Indo-Pacific Fish. Coun.* No. 59/12
- Smith O R 1947 Returns from natural spawning of cut throat trout and eastern brook trout; *Trans. Am. Fish. Soc.* 74 281-296
- Varghese T J 1961 Observations on the biology of *Raonda russelliapa* (Gray); *Indian J. Fish.* 8 96-106
- Varghese T J 1973 The fecundity of the rohu, *Labeo rohita* (Ham.); *Proc. Indian Acad. Sci.* 77 214-224
- Varghese T J 1976 Studies on the fecundity of *Coilia ramcarati* (Ham-Buch); *Proc. Indian Acad. Sci.* B83 47-54

Bionomics of hill-stream cyprinids. III. Food, parasites and length-weight relationship of Garhwal mahaseer, *Tor tor* (Ham.)

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Abstract. 829 *Tor tor* (Ham.) were examined for food habits, parasites and length-weight relationships. Parabolic equations describing the body length-body weight relationships were $W = 0.0009298 L^{2.0553}$, $W = 0.0013146 L^{1.9769}$, and $W = 0.0010884 L^{1.9561}$ for females, males and pooled fishes respectively. The regression coefficients of the < 15.0 cm, 15.1-20.0 cm and > 20.1 cm length classes and sexes were found to be significantly different from one another and from 3. The regression coefficients of the fishes of larger size classes were higher than those of the fishes of < 15.0 cm size classes.

Keywords. Gut contents; parasites; length-weight relationship; regression coefficient; parasitocoenosis; variance; Himalayan riverine ecosystem.

1. Introduction

Garhwal mahaseer, *Tor tor* (Ham.), is of economic value in the hilly area and it is available almost throughout the year in the rivers of Garhwal Himalayas. The present investigation was conducted to help fill the need for more information on the general biology of this fish in the area. It deals with the bionomics and helminthocoenoses of *T. tor* in Garhwal Himalayas; this study is also part of an investigation into the biology and fishery of hill-stream fishes, results of certain aspects of which have already been published by the author and coworkers (Malhotra 1981a, b; Malhotra (in press); Malhotra *et al* 1980a, b).

2. Material and methods

Methods of collection of samples and their analyses were published earlier (Malhotra 1981a; Chauhan *et al* 1981). 829 *T. tor* of 4.5-79 cm length range (with one fish measuring 125 cm) were used in the present investigation. The length-weight relationship was estimated by the formula,

$$W = aL^b$$

where W = weight, L = body length and a and n are constants. Logarithmic transformation of this may be written as :

$$\log W = \log a + n \log L$$

where, $\log W$ is the dependent variable (Y), $\log L$ the independent variable (X), n the regression coefficient or slope (b) ; and $\log a$ the Y -intercept. Analysis of variance (Snedecor and Cochran 1967) was applied and the coefficient of determination (r^2) (Croxtton 1953) and the values of least squares regression slopes (Zeller and Carmines 1978) were computed.

3. Results

3.1. Food

Qualitative and quantitative (percentage by weight) analysis of gut contents including food and parasites showed 5.49% worms, 8.42% *Cladophora* sp., *Spirogyra* sp., *Sphaerocystis* sp., *Volvox* colonies and plant debris and 86.09% insects, their larvae and nymphs, viz., coleopterans (*Corixa* sp., *Psephenus* sp.), dipterans (*Tendipes* sp.), hemipterans (*Gerris* sp.), trichopteran larvae, ephemeropteran nymphs (*Heptagenia* sp.) and plecopteran nymphs.

3.2. Parasites

The frequency of parasites in alimentary canal of examined fishes was 0.20% cestodes, 99.50% nematodes, and 0.30% trematodes. *Bothriocephalus teleostei* (Malhotra 1981b) was the only cestode and *Diplostomum minimum* was the only trematode recorded from the small intestine. However, 79.72% of the nematodes collected were females and 20.28% were males. Out of these 8.09% female and 11.36% male specimens of *Pseudanisakis* sp. were gathered from stomach while 61.85% female ; 50.0% male specimens of *Comephronema* sp. and 30.06% female ; 38.64% male specimens of *Cystidicoloides* sp. were collected from small intestine.

3.3. Length-weight relationship

The ratio of total and standard length of fish including body weight have been computed in table 1. It illustrates a comparative account of various relationships between different body measurements and body weight.

3.4. Estimated regressions

Altogether 829 fish of the length range 4.5–79 cm (with one fish of 125 cm) were analysed. An initial assessment suggested that the same equation would not fit the data for the entire length range and that breaks occurred around 10.0–15.0 cm ; 15.1–20.0 cm ; and > 20.1 cm groups. Separate parabolic equations, their logarithmic transformations, and linear regression were, therefore, computed for different groups as mentioned in table 2.

The significance of differences between the regression coefficients (b) was tested by the method of analysis of variance. The relevant data have been presented in table 3.

Table 1. Mean values of body weight and ratios of total/standard lengths of *Tor tor* (Ham.).

Sample size	Mean \pm S.E.				
	Total length (cm)	Standard length (cm)	TL/SL ratio	Body weight (g)	
Female	474 19.4 \pm 0.6691	16.0109 \pm 0.5921	1.2088 \pm 0.001	272.8	\pm 52.169
Male	355 18.5 \pm 0.4739	15.4746 \pm 0.4345	1.2051 \pm 0.0034	131.7	\pm 27.3614
< 15.0 cm	549 12.5593 \pm 0.0923	10.8927 \pm 0.0858	1.1530 \pm 0.1131	27.2993	\pm 0.7517
15.1-20.0 cm	149 17.0639 \pm 0.1168	17.6628 \pm 0.1062	1.3458 \pm 0.1342	86.0302	\pm 6.1856
> 20.1 cm	131 31.8632 \pm 1.1824	34.5254 \pm 1.5788	1.6186 \pm 0.2459	1155.6406	\pm 185.1899
Pooled	829 19.0041 \pm 0.4349	15.7957 \pm 0.3864	1.2017 \pm 0.0029	212.1402	\pm 32.1309

Table 2. Regression equations describing length-weight relationship in *Tor tor* (Ham.).

Category	Logarithmic regression equations	Parabolic equations
Female	$\log W = 3.0316 + 2.0553 \log L$	$W = 0.0009298 L^{2.0053}$
Male	$\log W = 2.8812 + 1.9769 \log L$	$W = 0.0013146 L^{1.9769}$
< 15.0 cm	$\log W = 1.4208 + 1.4819 \log L$	$W = 0.037949 L^{1.4819}$
15.1-20.0 cm	$\log W = 2.1459 + 2.00 \log L$	$W = 0.0071466 L^{2.00}$
> 20.1 cm	$\log W = 3.4281 + 2.4156 \log L$	$W = 0.0003732 L^{2.4157}$
Pooled	$\log W = 2.9632 + 1.9561 \log L$	$W = 0.0010884 L^{1.9561}$

Table 3. Analysis of covariance between the regression coefficients (*b*) for *Tor tor* (Ham.).

N	Female 474	Male 355	Pooled 829	< 15 cm 549	15.1-20.0 cm 149	> 20.1 cm 131
$\Sigma (X - \bar{X})^2$	5.0027	4.4227	5.1108	3.3451	2.4386	4.6242
$\Sigma (Y - \bar{Y})^2$	8.7873	7.9748	8.8412	5.0822	5.9265	8.7579
$\Sigma (X - \bar{X})(Y - \bar{Y})$	6.8447	6.1060	6.8854	4.0375	3.5456	6.6699
$b\Sigma (X - \bar{X})(Y - \bar{Y})$	14.0678	12.0710	13.4686	5.9832	7.0912	16.1118
σ^2 unexp.	1.6427	1.415	1.7228	0.3504	0.2417	1.5074
ρ^2	0.7931	0.6512	0.6607	0.4443	0.0532	0.8999
r^2	0.7949	0.6101	0.6397	0.3158	0.0579	0.8684

ρ^2 = Proportion of correlated variance ; σ^2 = Unexplained variance.

The test of heterogeneity of regressions is given below :—

Source of variation	df	Sum of squares	Mean square	F
Between length classes :				
Deviation from average total regression	829	0.5725573		
Deviation from individual regression within sample	825	0.4380454	0.000530964	
Difference	4	0.1345119	0.0336279	63.33 $F_{0.5\%} = 3.72$
Between sexes				
Deviation from average total regression	829	0.0054845		
Deviation from individual regression within sample	825	0.0037392	0.0000045	
Difference	4	0.0017453	0.0004363	96.96 $F_{0.5\%} = 3.72$

The differences between the regression coefficients were significant at 0.5% level.

A comparison of the regression lines of the length-weight relationship of *T. tor* has been presented in table 4. According to the standardized least squares linear regression, for each standard unit of length, the fish gained 0.890–0.891 ; 0.799–0.836 ; 0.806–0.820 ; 0.562–0.791 ; 0.220–0.242 ; and 0.947–0.950 of a standard unit of weight for females (size group 6–78 cm and one fish of 125 cm); males (size group 4.5–79 cm) ; pooled ; < 15.0 cm ; 15.1–20.0 cm ; and > 20.1 cm groups of *T. tor* respectively. In both the sexes *r* is significant.

A logarithmic plot of weight (mean values) on length (mean values in 5.0 cm length intervals in 829 fishes and the linear regression for separate groups and pooled fishes are shown in figure 1.

4. Discussion

4.1. Food and parasites

The analysis of food reveals that *T. tor* is a carnioomnivorous fish but predominantly exhibits carnivorous habit. Nematode (*N*) parasites were more prevalent (99.50%) than cestodes (*C*) (0.20%) and trematodes (*T*) (0.30%). Hence a relationship $C < T < N$ could be established for *T. tor*. A detailed analysis of trends in parasitocoenoses in *T. tor* has been dealt with by Malhotra (in press) recently.

Table 4. Comparison of the regression lines of the length-weight relationship of *Tor tor* (Ham.)

Sample size	Variance		Covariance	Standardized least squares regression slope predicting		Level of significance r (P)
	Length	Weight		X from Y	Y from X	
Female (474)	2·3269	6·1116	4·1689	0·8897	0·8914	0·8916 (P < 0·025)
Male (355)	1·8724	5·4245	3·5558	0·7794	0·8355	0·7811 (P < 0·100)
< 15·0 cm (549)	0·6055	2·3427	1·2979	0·7905	0·5619	0·5620 (P < 0·250)
15·1–20·0 cm (149)	0·2654	3·75342	1·3724	0·2422	0·2197	0·2408 (P < 0·500)
> 20·1 cm (131)	2·5069	6·6441	4·5526	0·9501	0·9471	0·9319 (P < 0·005)
Pooled (829)	2·1922	5·9226	3·9669	0·8201	0·8057	0·7998 (P < 0·100)

4.2. Length-weight relationship

In the present investigation no major difference was found in the ratio value of total *vis-a-vis* standard length from that reported by earlier workers. There was a highly significant correlation of body length to body weight for female ($P < 0.025$), male ($P < 0.100$), pooled ($P < 0.100$) and > 20.1 cm length classes ($P < 0.005$) of *T. tor* (table 4). Based on the coefficient of determination (r^2) (Croxtan 1953), more than 79% of the variation in weight in females, 61% in males, 63% in pooled, and 86% in > 20.1 cm length class was attributable to the variation in length of the Garhwal mahaseer. However, only 31.58% and 0.06% of the variation in weight in < 15.0 cm and 15.1–20.0 cm length classes respectively was attributable to the variation in length of fish. Similarly the proportion of correlated variance (ρ^2) suggests that 79.31% variance in length in females, 65.12% in males, 66.07% in pooled fishes and 89.99% in fishes of > 20.1 cm length class was associated with weight while only 44.43% and 5.32% variance in length in fishes of < 15.0 cm and 15.1–20.0 cm length classes, respectively, was associated with weight. The length-weight relationship for female, male, pooled and fishes of < 15.0 cm, 15.1–20.0 cm and > 20.1 cm length classes of *T. tor* is defined and illustrated in figure 1.

The differences in regression coefficients between male and female fishes have been reported by Sekharan (1968), Eggleston (1970) and Krishnamoorthi (1971). The results of the present investigation show closeness to these studies in describing a significant difference between regression coefficients of different size classes and the sexes. It, however, does not conform to the views of Sekharan (1968) who regarded in *Sardinella albella* and *S. gibbosa*, higher values of regression coefficients in smaller length classes than in larger ones. Contrary to this, in the present study, the fishes of larger length classes, viz., 15.1–20.0 and > 20.1 cm

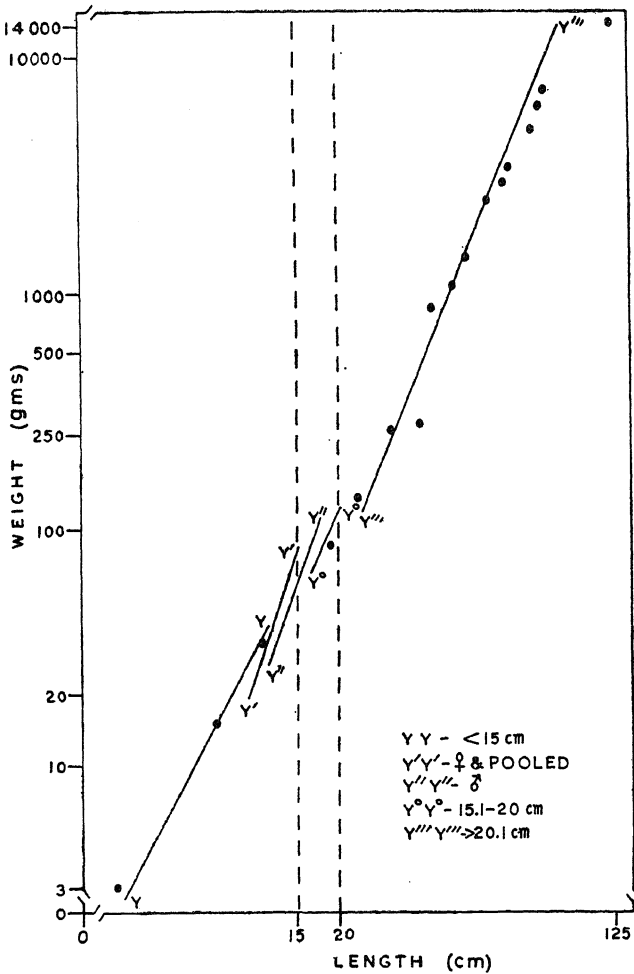


Figure 1. Length-weight relationship in *Tor tor* (Ham.).

showed higher values of regression coefficient ($b = 2.00-2.4156$) than the smaller ones (< 15.0 cm). This perhaps indicates a relatively rapid change in body outline of the fishes of 15.1 cm to 79 cm size group including 125 cm long fish, when they increase more in length than those of the fishes of smaller size classes.

As 'a' depends upon the obesity of the fish (LeCren 1951), by comparing the 'log a' values it is evident that the general fatness in the two sexes shows no significant difference in the present study like those reported by Narsimham (1970), Mojumdar (1971) and Vinci and Nair (1974). 'Log a' values also show appreciable difference in general fatness of individuals of different length classes contrary to the report of Sekharan (1968).

In this paper as per requirements of the exponential formula ($W = a L^b$) there was a consistently significant correlation in length and weight of *T. tor*. The values of regression coefficients indicate that the growth rate is lesser than the cube of length and represent an isometric trend (figure 1). Significant departures

from the isometric growth value have been reported by Narsimham (1970), Vinci and Nair (1974), Qadri and Mir (1980) and Malhotra (unpublished). This departure is statistically tested for the significance of the difference of the regression coefficient from 3. The regression coefficient and its standard error for the general relationship being 1.986 and 0.133 respectively, 't' test ($t = -7.61$ obtained by subtracting 3 from the regression coefficient and dividing the result by S.E.) indicated a high degree of significance, showing that the cubic law ($W = CL^3$; W = weight, L = length, C = constant) does not hold good for *T. tor* in the Himalayan riverine ecosystem.

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References

- Chauhan R S, Malhotra Sandeep K and Capoor V N 1981 The distribution and abundance of cestodes in eleven species of teleosts from Garhwal Himalayas with a note on host biology; *Himalayan J. Sci.* **1** 15-30
- Croxtan F E 1953 *Elementary statistics with applications in medicine and the biological sciences* New York: Dover Publ., pp. 376
- Eggleston D 1970 *A symposium on the Japan current* (ed.) J C Mar pp. 417-424
- Krishnamoorthi B 1971 Length-weight relationships for *Nemipterus japonicus* of Andhra-Orissa coast; *Indian J. Fish.* **18** 1-21
- LeCren E D 1951 The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*); *J. Anim. Ecol.* **20** 201-219
- Malhotra Sandeep K 1981a Cestode infection in freshwater fishes of Garhwal Himalayas, India; *Geobios.* **8** 90-92
- Malhotra Sandeep K 1981b Systems models for parasite pathways in ichthyoparasitology of the Himalayan riverine ecosystem; *Curr. Sci.* **50** 874-875
- Malhotra Sandeep K (in press) Trends in ichthyoparasitology of *Tor tor* (Ham.) in the Himalayan riverine ecosystem; *J. Himalayan Stud. Reg. Dev.*
- Malhotra Sandeep K, Chauhan R S and Capoor V N 1980a Nematode infection in relation to some ecological aspects of hill-stream fishes; *Geobios.* **7** 193-198
- Malhotra Sandeep K, Chauhan R S and Capoor V N 1980b Statistical analysis of cestode, infection in relation to some ecological aspects of hillstream fishes in Garhwal Himalayas, India; *Indian J. Helminthol.* **32** 43-52
- Mojumdar P 1971 Length-weight relationship in the cat-fish *Tachysurus thalassinus* (Ruppell), *Indian J. Fish.* **18** 179-182
- Narsimham K A 1970 On the length-weight relationship and relative condition in *Trichiurus lepturus* Linnaeus; *Indian J. Fish.* **17** 90-96
- Qadri M Y and Mir S 1980 Length-weight relationship of *Orienus plagiostomus* (McClell.); *Geobios* **7** 158-159
- Sekharan K V 1968 Length-weight relationship in *Sardinella albella* (Val.) and *S. gibbosa* (Bleek); *Indian J. Fish.* **15** 166-174
- Snedecor G W and Cochran W G 1967 *Statistical Methods* (6th edn) (Ames, Iowa: Iowa State Univ. Press) pp. 593
- Vinci G K and Nair A K K 1974 Length weight relationship in the threadfinbream, *Nemipterus japonicus* along the Kerala coast; *Indian J. Fish.* **21** 299-302
- Zeller R A and Carmines E G 1978 *Statistical analysis of social data* (Chicago; Rand McNally College Publ. Co.) pp. 398

Effects of sublethal levels of DDT, malathion and mercury on tissue proteins of *Sarotherodon mossambicus* (Peters)

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Abstract. Liver and muscle total proteins declined in *Sarotherodon mossambicus* subjected to sublethal concentrations of DDT, malathion and mercury. The results indicate their role in maintenance of energy supply irrespective of the nature of the toxicant. The qualitative variations in serum protein pattern also support the quantitative changes in tissues.

Keywords. Toxic stress ; proteolysis ; iso-osmotic ; milieu interior.

1. Introduction

Tissue total proteins as energy sources for fishes during thermal stress, spawning and muscular exercise have been demonstrated by several investigators (Fontaine and Hatley 1953 ; Idler and Clemens 1959). However, studies on the impact of toxicants on tissue energy sources are relatively few, though considerable information is available dealing with the determination of acute toxic levels of several pollutants and their influence on oxidative metabolism. In this paper, an attempt has been made to determine the extent of changes in the level of proteins in two principal tissues, liver and muscle and also the electrophoretic pattern of serum proteins in the fish *Sarotherodon mossambicus* exposed to sublethal concentrations of DDT, malathion and mercury.

2. Materials and methods

Sarotherodon mossambicus (Peters) (15-20 g) were obtained from local ponds maintained by Tamil Nadu state fisheries research station, and acclimated in the laboratory for 15 days. They were fed with cooked rice mixed with dried prawn powder. DDT (III-Trichloro 2-2-Bis (P-Chlorophenyl ethane) as 10% wettable powder and malathion (S-1,2 Bis (ethoxy-carbonyl) ethyl *o*, *o*-dimethyl phosphorodithiate) as 5% wettable powder, supplied by M/s Parry and Company Limited,

Madras, were employed for the sublethal tests. The chloride form of mercury (HgCl_2) was used as the heavy metallic compound. Acetone was used as the solvent for DDT and water for both malathion and mercury. Two sets of fishes each consisting of five were exposed to 0.01 ppm of DDT, 0.95 ppm of malathion and 0.09 ppm of mercury, the respective sublethal levels representing the active ingredients of the toxicants. The sublethal concentrations of them were calculated by multiplying an application factor of $0.25 \times$ with the respective LC 50 values determined from the acute toxicity tests, as recommended by the Ontario Ministry of Environment (1974). The fishes were exposed for the 24 hr, 7 days and 15 days simultaneously along with controls for each. At the end of respective intervals, fishes were sacrificed and tissues were taken for total protein analysis. The protein was estimated following the procedure of Lowry *et al* (1951). For the qualitative study of serum proteins, disc electrophoresis using polyacrylamide gel was carried out. The pattern of fractions obtained after 15 days exposure is indicated in figure 3.

3. Results

The levels of total protein in the liver and muscle of control and toxicant exposed groups are shown in figures 1 and 2. There appears to be no significant difference either in the liver or muscle of the control and the three toxicant exposed groups at 24 hr interval. However, a significant decrease was noticed after 7 and 15 days in both tissues ($P = 0.05$). Electrophoretic studies revealed that serum proteins in fishes kept under control showed eleven fractions. On the contrary, in fishes exposed to DDT—a total of fourteen fractions, and in those exposed to malathion and mercury, ten and nine fractions were discernible respectively.

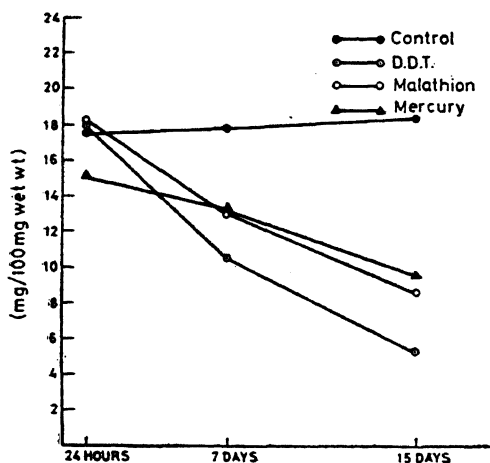


Figure 1. Total protein (liver) (mg/100 mg wet wt.).

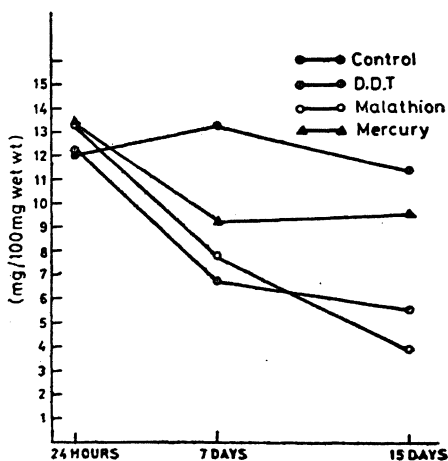


Figure 2. Total protein (muscle) (mg/100 mg wet wt.).

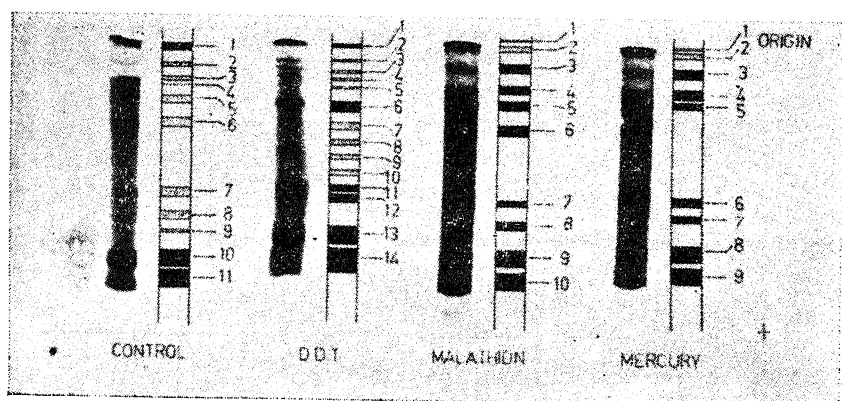


Figure 3. Polyacrylamide-gel-electrophoretic patterns of serum proteins of control group vs. toxicant-exposed groups.

4. Discussion

The total proteins in the liver and muscle showed a steady decline after 7 and 15 days, in contrast to 24 hrs interval. The absence of considerable alterations in the total protein content during the initial period of exposure (24 hrs) supports the concept of Fry (1971) that fishes tend to resist a changed situation for a specific period, but will eventually succumb as a result of their inability to continuously adapt. The pattern of changes in the total carbohydrates in blood, the free sugars in liver and muscle and the consequent depletion of glycogen in these tissues at the initial period of exposure (24 hr) in this animal (Ramalingam 1980) also lends support to the view extended by Umminger (1970) that carbohydrates represent the principal and immediate energy precursors for fishes exposed to stress conditions while proteins being the energy source to spare during chronic periods of stress.

Depletion of tissue proteins in fishes exposed to various toxicants has been reported by several investigators (McLeay and Brown 1974; Sakaguchi and Hamaguchi 1975; Shakoori *et al* 1976). Besides the above changes, the protein fractions in the serum of fishes exposed to toxicants, revealing an increase in the case of DDT while a decrease in malathion and mercury-exposed ones also indicate the conversion of tissue proteins into soluble fractions reaching the blood for utilisation. Similar qualitative changes have been reported by Anees (1974) in *Channa punctatus* exposed to diazinon, dimethoate and methyl parathion for 14 days.

The decline in the liver and muscle protein would suggest an intensive proteolysis which in turn could contribute to the increase of free aminoacids to be fed into the tricarboxylic acid cycle (TCA) as keto acids, thus supporting the hypothesis of Kabeer Ahamad *et al* (1978). Such a possibility is further strengthened by the investigations of Shaffer (1967)—Mehrle *et al* (1971), Shakoori *et al* (1976) which revealed both qualitative and quantitative variations in the tissue aminoacids of fishes exposed to toxicants. In addition, studies of Bell (1968), McKim *et al* (1970), Lane and Scura (1970), Sakaguchi

and Hamaguchi (1975) have also revealed marked variations in the activity of enzymes involved in transaminations in fishes at similar situations. However, an understanding of the levels of aminoacids at different intervals during stress imposed by toxicants, would be of interest in explaining the role of tissue proteins either to meet the energy demand completely or to maintain an iso-osmotic condition of the milieu interior also by increasing the aminoacids pool as suggested by Kabeer (1979).

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References

- Anees M A 1974 Changes in starch gel electrophoretic pattern of serum proteins of a freshwater teleost *Channa punctatus* (Bloch) exposed to sublethal and chronic levels of three organophosphorous insecticides; *Ceylon J. Sci.* **11** 53
- Bell G R 1968 Distribution of transaminases (aminotransferases) in the tissues of Pacific salmon (*Oncorhynchus*) with emphasis on the properties and diagnostic use of glutamic-oxaloacetic transaminase; *J. Fish. Res. Bd. Can.* **25** 1247-1268
- Fontaine M and Hatley J 1953 Contribution al etude du metabolisme glucidique du salmon *Salmo salar* L. a liver ses etapes de son developement et de ses migrations; *Physiol. Comp. Et Oecologia* **3** 37-52
- Fry F E J 1971 In *Fish Physiology* (eds.) W S Hoar and D J Randall (New York: Academic Press) Vol. 6 p. 1.
- Idler D R and Clemens W A 1959 The energy expenditures of Fraser river sockeye salmon during spawning migration to Chilke and Stuart lakes; *Int. Pacific. Salmon Fish. Comm. Prog. Rep.* Jackson Printing New Westminster B C 80 pp
- Kabeer Ahamad I, Begum Md, Sivaiah S and Ramana Rao K V 1978 Effect of malathion on free aminoacids, total proteins, glycogen and some enzymes of pelecypod *Lamellidens marginalis* (Lamarck); *Proc. Indian Acad. Sci.* **87** 377-381
- Kabeer Ahamad Sahib I 1979 Studies on some aspects of protein metabolism and associated enzyme systems in the freshwater teleost *Tilapia mossambica* to malathion exposure. Ph.D. Thesis, Sri Venkateswara University, Tirupati
- Lane G E and Scura E D 1970 Effects of dieldrin on glutamic oxaloacetic transaminase in *Poecilia latipinna*; *J. Fish. Res. Bd. Can.* **27** 1869-1871
- Lowry D H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with the Folin phenol reagent; *J. Biol. Chem.* **193** 265-275
- McLeay D J and Brown D A 1974 Growth stimulation and biochemical changes in juvenile coho salmon (*Oncorhynchus kisutch*) exposed to bleached kraft pulpmill effluent to 200 days; *J. Fish. Res. Bd. Can.* **31** 1043-1049
- McKim J M, Christensen G H and Hunt E P 1970 Changes in the blood of brook trout (*Salvelinus fontinalis*) after short term and long term exposure to copper; *J. Fish. Res. Bd. Can.* **27** 1883-1889
- Mehrle P M, Stalling D L and Bloomfield R A 1971 Serum aminoacids in rainbow trout (*Salmo gairdneri*) as affected by DDT and dieldrin; *Comp. Biochem. Physiol.* **38** 373-377
- Ontario Ministry of the Environment 1974 *Guidelines and criteria for water quality management in Ontario*. Supervised for publication by the Hon William G Newsman, Minister and Everest Biggs, Deputy Minister, 135 St. Clair Avenue, West Toronto
- Ramalingam K 1980 Studies on the effects of sublethal concentrations of a few toxicants on Biochemistry, Physiology and Histology of *Tilapia mossambica* (Peters). Ph.D. Thesis, University of Madras, Madras

- Sakaguchi H and Hamaguchi A 1975 Physiological changes in the serum and hepatopancreas of yellow tail injected with carbon tetrachloride; *Bull. Jpn. Soc. Scient. Fish.* **41** 283-290
- Schafer R 1967 The effects of pollutants on the free aminoacid content of the fish *Leuciscus cephalus* (L.) albus; *Rev. Biol.* **59** 385-407
- Shakoori A R, Saleem A Z and Muhammed S A 1976 Effect of malathion, dieldrin and endrin on blood serum proteins and free aminoacids pool of *Channa punctatus* (Block); *Pak. J. Zool.* **8** 124-134
- Umminger B L 1970 Physiological studies on supercooled killifish fundulus heteroclitus. III. Carbohydrate metabolism and survival at subzero temperature, *J. Exp. Zool.* **173** 159-174

Effect of teleostean prey size and salinity on satiation amount, satiation time and daily ration in the glassy perchlet *Chanda* (= *Ambassis*) *thomassi* (Day) (Pisces : Centropomidae)

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Abstract. Results of the experiments conducted to estimate the maximum single food intake, satiation time and daily ration in the predator, *Chanda thomassi* using different size groups of teleostean prey (guppies) and in six non-lethal salinities are presented. The results suggest that satiation amount and satiation time vary considerably with the size of the fish prey. It is seen that the appetite of the fish is lost on consuming relatively fewer number of larger fish prey, while the predator could accommodate a much larger number of smaller prey fish of greater gross size. Also the satiation amount decreases when the prey is available in bulk than when given at regular intervals. The computed daily ration of the predator shows high values when compared with available data on other tropical predators. The over all results project the destructive potential of this predatory species coupled with its shoaling habits.

Keywords. Satiation ; teleostean prey ; predator ; *Chanda thomassi*.

1. Introduction

Within the genetic potential of any species to grow, many abiotic and biotic factors limit maximum growth. The daily rate at which food can be consumed is a prime factor. This in turn is related to the capacity of the stomach (satiation feeding) and the rate of digestion. Thus, knowledge of food consumption in fish populations is, therefore, essential for interpretation of the influence of a variety of factors on fish production (Warren *et al* 1964 ; Windell 1966 ; Brocksen *et al* 1968 ; Brett *et al* 1969 ; Swenson and Smith 1973).

Information on the satiation amount i.e., the amount of food necessary to satisfy the fish (Brett 1971), the satiation time (time to attain satiation), and details regarding daily ration, and the gastric evacuation rates of a piscivorous predator are essential prerequisites for assessing the feeding capacity of these predators on valuable fish fry and fingerlings in the natural waters and culture systems. *Chanda* (= *Ambassis*) *thomassi* (Day) is a medium sized piscivorous predator found in shoals in the fresh and low saline waters of Kerala in South

India. With a view to estimating the satiation amount, satiation time and daily ration in the case of *C. thomassi* adults under laboratory conditions, a series of tests were conducted using four different size groups of the fish prey (*Poecilia* (= *Lebistes*) *reticulata* Peters) and six different salinities.

2. Materials and methods

Healthy individuals of *C. thomassi* immature adults (4.250 ± 0.250 g and standard length (SL) 7.1 ± 0.5 cm) were acclimated and reared in large plastic buckets (20 litre capacity). The temperature of the water was $27 \pm 1^\circ \text{C}$ and the oxygen content maintained at air saturation level. The fish were fed with an excess amount of fry, juveniles and adults of *Poecilia reticulata* for nearly fifteen days prior to experiments. The prey fish (*P. reticulata*) were then grouped into 4 size groups :—

- Group I fry (average SL 8 mm, average Wt. 16.3 mg)
- Group II juveniles (average SL 14.2 mm, average Wt. 57.0 mg)
- Group III mature males (average SL 18.6 mm, average Wt. 98.4 mg)
- Group IV mature females (average SL 24.8 mm, average Wt. 175.4 mg)

All the prey fish of each size group were almost of the same size and weight and the averages were calculated after weighing and measuring more than 50 fish collected at random from each group. Preliminary tests were conducted to find out the feeding intervals for each size group and the rate of feeding at each interval. They were estimated as 2 min and 5 fish (Group I), 5 min and 3 fish (Group II), 7 min and 3 fish (Group III) and 10 min and 2 fish (Group IV).

(1) At intervals (Expt. I)—The individuals of *C. thomassi* were starved for two days prior to the experiment in order to effect complete stomach evacuation. The precalculated numbers of prey fish were presented during each time interval removing the excess until the fish completely stopped feeding. To accommodate an initial high rate of feeding (Brett 1971), food was presented twice as fast during the first time interval. Fish were considered satiated when they would no longer accept any food, in the presence of excess, after a period of active feeding. The time from start to voluntary cessation is defined as the satiation time. Each experiment was done in triplicate.

(2) As a bulk (Expt. II)—Another experiment was done after a days starving presenting each fish with a bulk of fish prey (more than twice the satiation amount of the previous experiment) of each size group at a single instant. The fish were considered satiated when they did not capture a prey for a fifteen minutes time lapse. The satiation time was considered as the time from the start of feeding to the time of the last feed. Rough estimates of daily ration were made from the results of these experiments.

(3) In different salinities (Expt. III)—Also the fish were reared in 6 precalculated non-lethal salinities (0.96‰, 6.83‰, 9.75‰, 12.69‰, 15.62‰) and were provided with a bulk of prey (Group II) and the total amount consumed during the first 25 min, up to 12 hrs and up to 24 hrs were noted so as to roughly estimate the daily ration at different salinities.

3. Results

The satiation time, satiation amount, satiation amount as percentage of predator body weight (wet) and the amount of food consumed per unit time for the different prey fish size groups (feeding at intervals and in bulk) are illustrated in figures 1 and 2. It can be clearly discerned that with the increasing prey fish size there

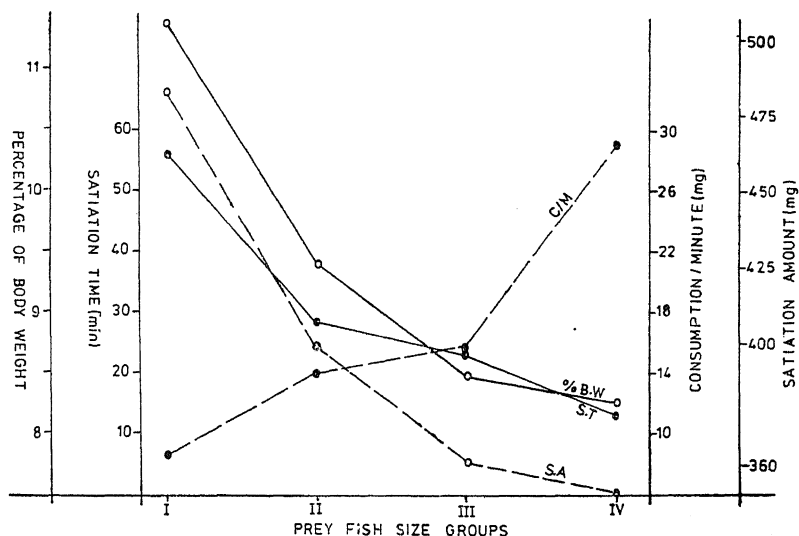


Figure 1. Effect of teleostean prey size on satiation amount, satiation time and consumption per unit time in *Chanda thomassi* when fed at intervals.

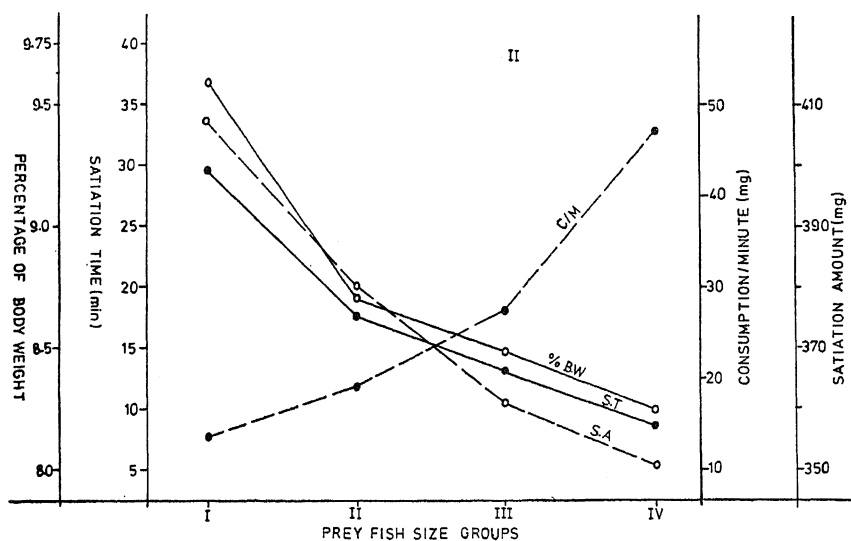


Figure 2. Effect of teleostean prey size on satiation amount, satiation time and consumption per unit time in *C. thomassi* when fed in bulk.

is a decline in satiation time, satiation amount and correspondingly in the amount consumed as percentage body weight whereas the amount of food consumed per unit time shows a steady increase. From the two experiments the satiation time ranges from 27–58 min and satiation amount from 391.2 mg–505.3 mg for group I, 15.0–30.0 min and 342.0–456.0 mg for group II, 10.5–28.0 min and 295.2–393.6 mg for group III and 6.0–20.0 min and 350.8 mg for group IV.

Taking into account only the light phase of the 24 hr day (the fish were found to rest on the bottom individually and not to feed during the night and to reshool and start feeding at dawn), and the time for 100% stomach evacuation (9 hrs),

Table 1. Effect of teleostean prey size on the daily ration of *C. thomassi* (immature adults) when fed at intervals and in bulk.

At intervals		In bulk	
Prey fish size groups	Daily ration as % wet body weight of predator	Prey fish size groups	Daily ration as % wet body weight of predator
Group I	22.76	Group I	19.18
Group II	18.78	Group II	17.88
Group III	16.98	Group III	16.98
Group IV	16.50	Group IV	16.50

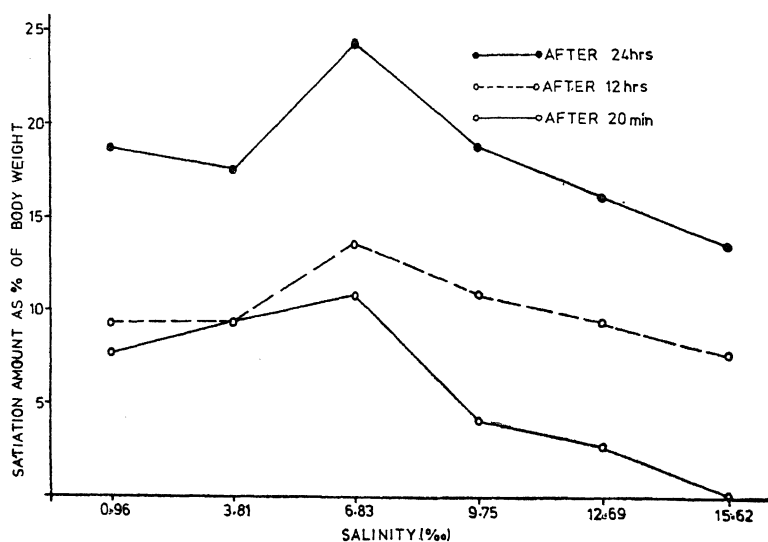


Figure 3. Effect of six non lethal salinities on the food consumption (25 min and 12 hrs) and daily ration of *C. thomassi*.

it was found safe to assume that active feeding is restricted mainly to the dawn and dusk (twice a day). Thus a rough estimate of the daily ration was made as twice the satiation amount and presented as percentage of the predator body weight in table 1.

The satiation amounts for the first 25 min, for 12 hrs and 24 hrs for the six different salinities are shown in figure 3. The highest amount of food intake in 24 hrs is shown to be at 6.83‰ S i.e., 24.14% of the predator body weight, the lowest amount being at 15.62‰ S i.e., 13.41% of the predator body weight. Thus in the 0.9‰–15.62‰ salinity range, a rough daily ration range of 13.41%–24.14% of the predator body weight is seen.

4. Discussion

The results suggest that the satiation time and satiation amount vary considerably with the size of the fish prey. The satiation amount and time are inversely proportional and the amount of food consumed per unit time is directly proportional to the size of the fish prey. It would appear that the appetite of the fish is lost on consuming relatively fewer number of larger fish prey, while the predator could accommodate a much larger number of small fish prey of greater gross size.

An analysis of the available information would indicate that (1) Stretch receptors in the stomach wall constitute one of the mechanisms controlling the appetite of vertebrates (Lepkovsky 1948 ; Paintal 1954). Consequently the size of individual particles would apparently determine the point at which further distention is declined. This will also be checked to some extent by the shape of the individual particles, especially in predators where the prey is swallowed as a whole as in the present study, so as to utilise the maximum space of the total available extended stomach volume. (2) Animals tend to eat to satisfy their energy demand so that the calorific content of the food will also affect the size of daily ration (Rozin and Mayer 1961).

The predator's maximum single intake (satiation amount) of the prey fish fry and juveniles (Groups I and II) decreases when the prey is available in bulk than at regular intervals (483.57 to 407.5 mg and 399.0 to 380.0 mg). Thus the predator may consume more food if the fry and juveniles of the prey fish form scattered groups being available to the predator as individuals at short intervals than when they are in abundance forming large tight-knit shoals.

Thus, it would appear that a 58 min feeding time with feeding at intervals, and 35 min feeding time with feeding in bulk would be quite adequate to satiate *C. thomassi* immature adults at $27 \pm 1^\circ \text{C}$ independent of the size of the fish prey. The Jack mackerel (*Trachurus japonicus*) and the rainbow trout (*Salmo gairdneri*) feeding on mackerel meat and 'compound feed' at 25° and 10°C , respectively, required 60 and 65 min to reach satiation, whereas two other species, the puffer (*Fugu vermicularis*) and the file fish (*Stephanolepis cirrhifer*) were satiated within 6 and 13 min respectively, indicating a marked species difference (Ishiwata 1968). Brett (1971) found the satiation time for three different sizes of the sockeye salmon, *Oncorhynchus nerka* varied from 33 to 50 min while feeding on 'Abernathy pellets' (Fowler and Banks 1969) at 15°C ,

The maximum single intake (satiation amount) for *C. thomassi* (4.250 ± 0.250 g) ranged from 8.25% to 11.38% of the predator wet body weight. Brett (1971) found that the amount of food in a full stomach of the sockeye salmon varied from 3 to 13% among the small fish (3 to 6 g) and from 1 to 5% among the larger fish (150–350 g).

The computed maximum daily intake (daily ration) from the two experiments and for the different salinities shows a range of 16.50 to 22.76% (for different prey size) and 13.51% to 24.14% (for different salinities) of the predator wet body weight at a water temperature of $27 \pm 1^\circ\text{C}$. In terms of single and daily maximum intake the smaller prey fish contributed to the maximum values and *vice versa*. The daily rations of the Cuban predaceous fish from the family Serranidae were 2.41–5.7% of the body weight during the summer at 28 to 29°C (Reshetnikov and Popova 1975 ; Reshetnikov *et al* 1975). According to Brett (1971), the total daily intake decreased from 16.9% of dry body weight for the 4 g fish to 4.3% for the 216 g fish (sockeye salmon) when fed on pellets. It is of interest to note that daily rations are highest in young fish during the transition to predation, 9 to 40%, an average of 21.9% of the body weight in new broods of 4 to 5 cms sheat fish, 9.50% in fry 5–7 cms and 7.7% in individuals 7 to 9 cm long (Popova 1978). The transition to a piscivorous feeding habit is during the late juvenile and immature adult stages in glassy perchlets and may be one of the reasons for the high values for daily ration obtained (22.76% and 24.14%) in the present study. Also in the extreme instance of a starving predaceous fish (starving for 2 days prior to feeding during the experiments), the pattern of feeding may lead to degrees of stomach distension that considerably exceed that of the maximum capacity exhibited by the daily particulate feeder. However, these results only give the maximum single and daily intake under laboratory conditions whereas the daily ration and maximum single intake will be different in the natural waters as the intensity of feeding of predaceous fish and their daily ration will change with the seasonal changes in ecological conditions, but it is important that during favourable periods they can attain these or higher values.

The results of the present study thus show the destructive potential this predator has in the form of high values of food intake (piscivorous). At the same time the study also reveals how the size of the prey fish and mode of feeding can be favourably manipulated in captivity to maximise the daily food intake and thereby promote growth in the culture of other predaceous fish.

Acknowledgements

One of us (JRN) is thankful to the Council of Scientific and Industrial Research, Government of India for a Research Fellowship during the tenure of which the present work was carried out.

References

- Brett J R 1971 Satiation time, appetite and maximum food intake of sockeye salmon (*Oncorhynchus nerka*) ; *J. Fish. Res. Bd. Canada* 28 409–415

- Brett J R, Shellbourn J E and Shoop C T 1969 Growth rate and body composition of fingerlings sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size ; *J. Fish. Res. Bd. Can.* **26** 2363-2394
- Brocksen R W, Davis G E and Warren C E 1968 Competition, food consumption and production of sculpins and trouts in laboratory stream communities ; *J. Wildl. Manage.* **32** 51-75
- Fowler L G and Banks J L 1969 Tests of vitamin suppliments and formula changes in Abernathy salmon diets, 1966-1967 ; *U.S. Fish. Wildl. Serv. Tech. Pap.* **26** 1-19
- Ishiwata N 1968 Ecological studies on the feeding of fishes IV. Satiation curve ; *Bull. Jpn. Soc. Sci. Fish.* **34** 691-693
- Lepkovsky S 1948 The physiological basis of voluntary food intake (appetite) ; *Advan. Food Res.* **1** 105-148
- Paintal A S 1954 A study of gastric stretch receptors. Their role in the peripheral mechanism of satiation of hunger and thirst ; *J. Physiol.* **126** 271-285
- Popova O A 1978 The role of predaceous fish in ecosystems ; in *Ecology of freshwater Fish Production* (ed.) S D Gerking (Oxford : Blackwell Scientific Publications) pp. 215-249
- Reshetnikov Yu S and Popova O A 1975 The daily rations and rate of food digestion in tropical fish ; *Sbornik 'Biologiya Shelfa'*, Vladyvostok, 144-145
- Reshetnikov Yu S, Sylva A, Claro R and Popova O A 1975 Rates of food digestion in tropical fishes ; *Zool. Zh.* **54** 1506-1514
- Rozin P and Mayer J 1961 Regulation of food intake in the gold fish ; *Am. J. Physiol.* **201** 968-974
- Swenson W A and Smith L L Jr 1973 Gastric digestion, food consumption, feeding periodicity and food conversion efficiency in Walleye (*Stizostedion vitreum vitreum*) ; *J. Fish. Res. Bd. Canada* **30** 1327-1336
- Warren C E, Wales J H, Davis C E and Doudoroff P 1964 Trout production in an experimental stream enriched with sucrose ; *J. Wildl. Manage.* **28** 617-660
- Windell J T 1966 Rates of digestion in the blue gill sunfish ; *Invest. Indiana Lakes and Streams* **7** 185-214

Studies on some *Tetracotyle* Fillipi (1859) metacercariae from fishes of Lucknow

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Abstract. Three unknown *Tetracotyle* metacercariae, collected from piscine host, have been described. *Tetracotyle pandei* n.sp., *Tetracotyle srivastavai* n.sp. and *Tetracotyle ramalingi* n.sp. were collected from the visceral organs and musculature of *Channa punctatus* (Bl.). They are characterised by the shape and position of pseudosuckers, shape of hold fast organ and hold fast gland, number and position of genital rudiment and pattern of reserve excretory system.

Keywords. *Tetracotyle pandei* n.sp.; *Tetracotyle srivastavai* n.sp.; *Tetracotyle ramalingi* n.sp.; metacercariae; *Channa punctatus* (Bl.).

*Tetracotyle pandei** n. sp

Host : *Channa punctatus* (Bl.)

Location : mesenteries and liver of infected host

Locality : Lucknow

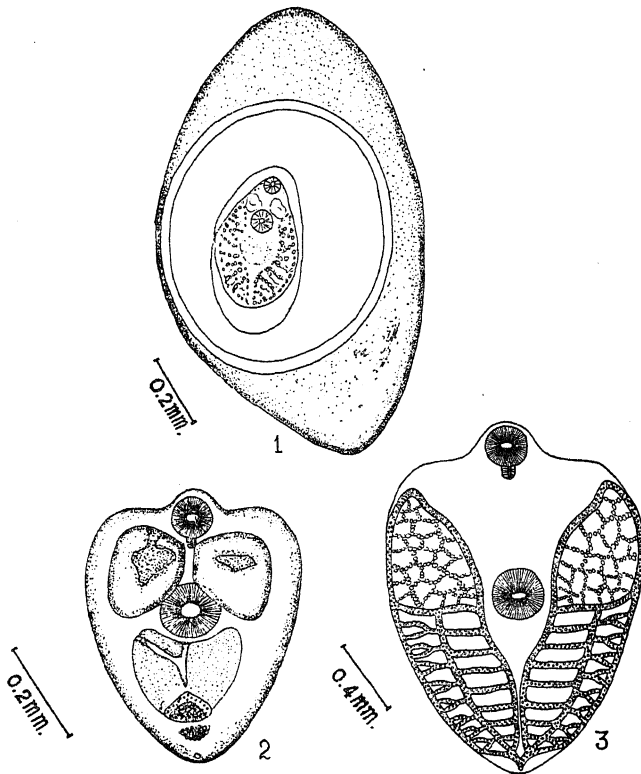
Number of host examined : 55

Number of host found infected : 3

Measurements in mm. Cyst— $1.22-1.24 \times 0.75-0.76$, outer layer— $1.22-1.24 \times 0.75-0.76$, middle layer— $0.66-0.69$ and inner layer— $0.44-0.46 \times 0.24-0.26$. Body— $1.80-1.82 \times 1.32-1.33$ (live) and $0.52-0.57 \times 0.40-0.50$ (fixed). Oral sucker— $0.13-0.14$ (live) and $0.07-0.08$ (fixed). Ventral sucker $0.19-0.20$ (live) and $0.09-0.10$ (fixed). Pharynx— $0.04-0.07$ (live) and $0.02-0.03$ (fixed). Pseudosucker— $0.15-0.18 \times 0.13-0.14$ (fixed). Hold fast organ— $0.08-0.09 \times 0.05-0.06$ (fixed).

Oval cyst (figure 1) three layered. Outer layer thick, fibrous, tough and pigmented, middle and inner layers thin. Body (figure 2) aspinose, with broad anterior and narrow posterior ends. Ventral sucker equatorial, larger. Large triangular pseudosucker one pair, posterior to oral sucker. Host fast gland triangular, deeply stained cell mass. Mouth terminal. Pharynx round and muscular.

* The species has been named in honour of Late Prof. B P Pande.



Figures 1-3. *Tetracotyle pandei* n.sp. 1. Encysted metacercaria (drawn from a live specimen). 2. Metacercaria (drawn from a fixed specimen). 3. Metacercaria showing reserve excretory system (drawn from a live specimen).

Oesophagus and intestinal caeca not visible. Genital rudiments two, anterior rudiment at the posterior border of hold fast organ, posterior rudiment in the posterior body region.

The excretory system (figure 3) of secondary reserve excretory system and a primary system of flame cells. "V" shaped excretory bladder at posterior end with terminal excretory pore giving rise to three pairs of canals, outer, middle and inner longitudinal canals. Each inner and outer longitudinal canals joined in the region of pseudosuckers, forming an isthmus of small canals. Median longitudinal canal, running up to the region of ventral sucker. Seven transverse canaliculae to inner longitudinal canal and eight bifurcated transverse canaliculae to outer longitudinal canal. Whole reserve excretory system filled with freely moving, round excretory corpuscles. Primary system of flame cells not observed.

Discussion

The present form chiefly differs from the other species in having three layered cyst and the pattern of reserve excretory system. It can be further differentiated from

T. ranae (Kaw 1950) in having a cyst, from *T. xenentodoni* (Chakrabarti 1970b) and *T. muscularis* (Chakrabarti 1970a) in the ratio of suckers, from *T. sophoriensis* (Singh 1956), *T. glossogobi* (Chakrabarti 1970c) and *T. tandoni* (Pandey 1973) in having an undivided body, from *T. indicus* (Singh 1956) by the number of genital rudiments, from *T. baughi* (Pandey 1973), *T. lymnaei* (Pandey and Agrawal 1978), *T. lucknowensis*, (Pandey 1971b) *T. lali* (Pandey 1971a), and *T. szidati* (Chakrabarti and Baugh 1970) in the position of pseudosuckers and shape of hold fast organ and *T. bufoi* (Agrawal 1975) in having a well-developed ventral sucker.

It, however, closely resembles with *Tetracotyle* of *Apatemon pellucidus* Yamaguti 1933 and *Tetracotyle* of *Apatemon fuligulae* Yamaguti 1933. It can be distinguished from *Tetracotyle* of *A. pellucidus* by the number of genital rudiments and from *Tetracotyle* of *A. fuligulae* in having two masses of genital rudiments and in the absence of prepharynx. It differs from *T. communis* and *T. diminuta* Hughes, 1928 in the pattern of excretory system and number of genital rudiments.

***Tetracotyle srivastavai* n. sp.**

Host : *Channa punctatus* (Bl.)

Location : mesenteries

Locality : Lucknow

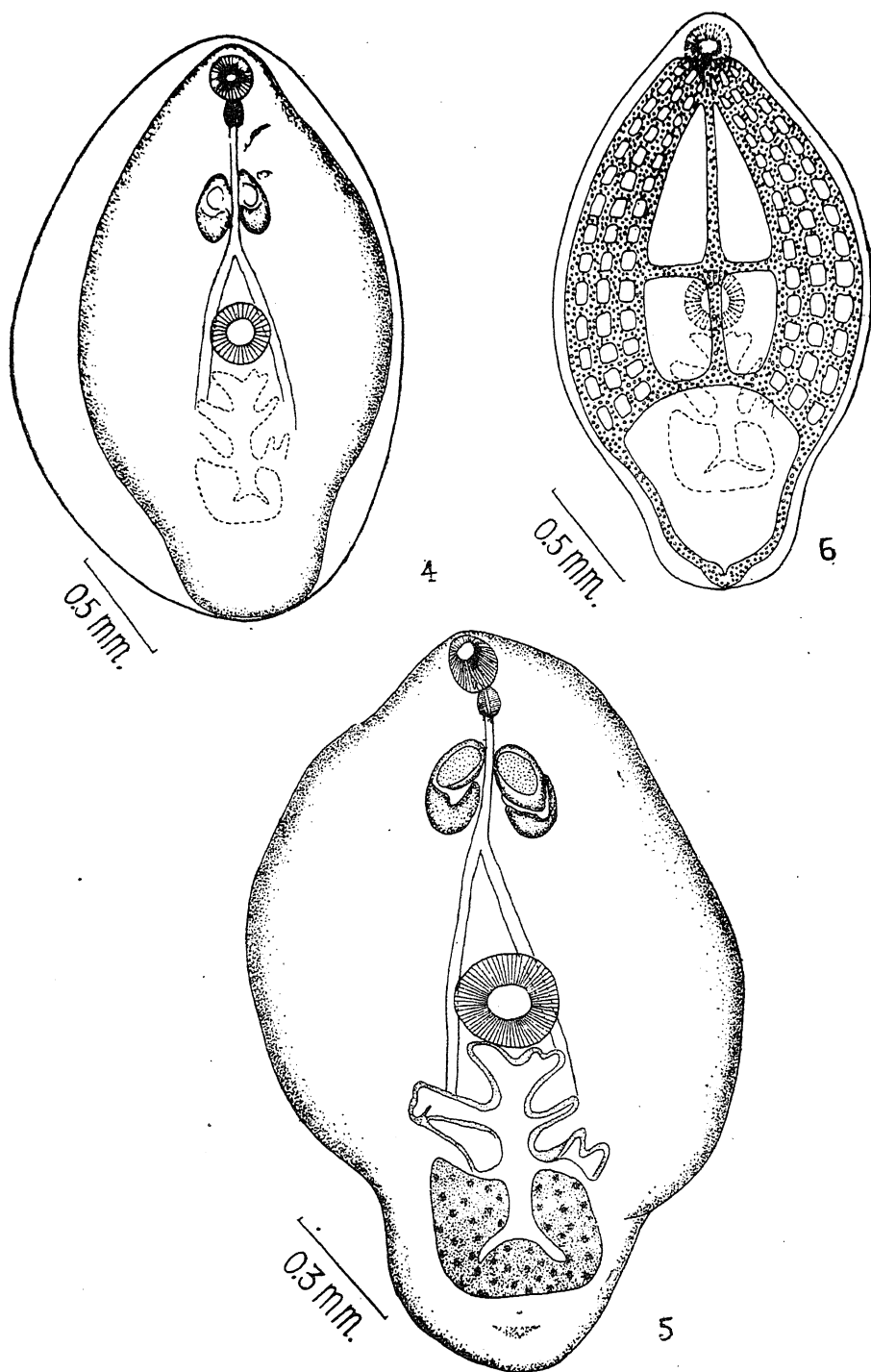
Number of hosts examined: 55

Number of hosts found infected: 2

Measurements in mm. Cyst—3.18–3.19 × 1.57–1.59 (live) and 1.68–1.70 × 0.84–0.85 (fixed). Forebody—2.01–2.03 × 1.37–1.39 (live) and 1.34–1.36 × 1.12–1.14 (fixed). Hindbody—0.44–0.45 × 0.77–0.79 (live) and 0.34–0.36 × 0.55–0.57 (fixed). Oral sucker—0.18–0.19 (live) and 0.08–0.09 (fixed). Ventral sucker—0.25–0.27 (live) and 0.16–0.18 (fixed). Pseudosuckers—0.44–0.45 × 0.14–0.16 (live) and 0.23–0.24 × 0.13–0.14 (fixed). Pharynx—0.09–0.10 × 0.07–0.08 (live) and 0.05–0.06 × 0.035–0.04 (fixed). Oesophagus—0.57–0.58 (live) and 0.30–0.31 (fixed). Hold fast organ—0.45–0.47 × 0.38–0.40 (live) and 0.30–0.32 × 0.29–0.31 (fixed).

Oval cyst (figure 4) thin, transparent, single layered, with colourless fluid. Aspinose body (figure 5) divided into large fore and small hind body. Ventral sucker larger, equatorial. Pseudosuckers muscular, oval, in oesophageal region. Mouth terminal. Pharynx oval and muscular. Intestinal caeca up to the hold fast organ. Hold fast organ elongated, multilobed, with prominent cavity. Hold fast gland "U"-shaped, posterior to hold fast organ. Small mass of genital rudiment in posterior body region.

Small excretory bladder (figure 6) "V"-shaped, at hind end of body. Two main longitudinal canals, from excretory bladder run anteriorly up to oral sucker. Two transverse canals, anterior and posterior, joined by three lateral longitudinal and one median longitudinal canal. Inner lateral longitudinal canals of two sides, joined together in ventral sucker region by a short, median transverse canal. Main longitudinal canal and three lateral longitudinal canals of each side, joined together by 10–14 short transverse canaliculae.



Figures 4-6. *Tetracotyle srivastavai* n.sp. 4. Encysted metacercaria (drawn from a live specimen). 5. Metacercaria (drawn from a fixed specimen). 6. Metacercaria, showing reserve excretory system (drawn from a live specimen).

Discussion

The present larva shows close resemblance with *T. ranae* Kaw 1950 ; *T. ujjainensis* Trivedi 1964 ; *T. muscularis* Chakrabarti 1970a ; *T. baughi* and *T. tandoni* Pandey 1973 and *T. lymnaei* Pandey and Agrawal 1978 in having a divided body. However, it differs from *T. ranae* and *T. ujjainensis* in the ratio of suckers, from *T. muscularis* in the ratio of suckers and genital rudiment, from *T. baughi* and *T. tandoni* in the number of genital rudiment and lobed hold fast organ and from *T. lymnaei* in the number of genital rudiment. It also differs from all the above species in having different pattern of reserve excretory system.

This form shows resemblance also with *T. communis* Hughes 1928 ; *Tetracotyle* of *A. pellucidus* Yamaguti 1933 and *Tetracotyle* of *A. fuligulae* Yamaguti 1933. However, it differs from *T. communis* in having lobed hold fast organ, from *Tetracotyle* of *A. pellucidus* in the number of genital rudiment and from *Tetracotyle* of *A. fuligulae* in having lobed hold fast organ and in the absence of a prepharynx.

Tetracotyle ramalingi n. sp.

Host : *Channa punctatus* (B1.)

Location : muscle fibres of infected host

Locality : Lucknow

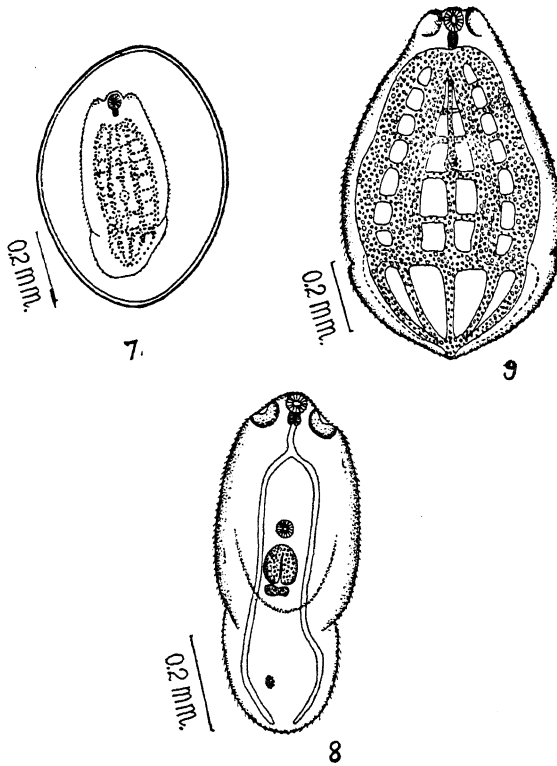
Number of hosts examined : 55

Number of hosts found infected : 2

Measurements in mm. Cyst—0.74–0.75 × 0.56–0.57 (live) and 0.40–0.43 × 0.37–0.38 (fixed). Forebody—0.70–0.72 × 0.60–0.62 (live) and 0.50–0.52 × 0.30–0.33 (fixed). Hindbody—0.30–0.32 × 0.58–0.60 (live) and 0.20–0.22 × 0.24–0.26 (fixed). Oral sucker—0.06–0.07 (live) and 0.04–0.05 (fixed). Ventral sucker—0.04–0.05 (live) and 0.025–0.03 (fixed). Pseudosuckers—0.08–0.09 × 0.05–0.055 (live) and 0.06–0.07 × 0.03–0.04 (fixed). Pharynx—0.04–0.05 × 0.03–0.04 (live) and 0.03–0.04 × 0.03–0.035 (fixed). Oesophagus—0.05–0.06 (live) and 0.03–0.04 (fixed). Hold fast organ—0.08–0.09 × 0.07–0.075 (fixed).

Cyst (figure 7) oval, thick and double layered. Outer layer thicker. Body (figure 8) oval, spinose and divided. Forebody larger. Oral sucker round, terminal and larger. Ventral sucker equatorial in forebody. Pseudosuckers lateral, muscular and kidney-shaped. Mouth leading to oval, muscular pharynx. Intestinal caeca up to posterior body region. Hold fast organ round to oval posterior to ventral sucker. Bilobed hold fast gland, close to hold fast organ. Single mass of genital rudiment in posterior hind body region.

Four longitudinal excretory canals (figure 9) from cornu of "V"-shaped excretory bladder, running anteriorly up to pharynx, joined anteriorly by anterior transverse canal, and posteriorly, by posterior transverse canal. Median longitudinal canal descending from anterior transverse canal up to posterior transverse canal. Further, longitudinal canals joined together by 5–8 transverse canaliculae.



Figures 7-9. *Tetracotyle ramalingi* n.sp. 7. Encysted metacercaria (drawn from a live specimen). 8. Metacercaria (drawn from a fixed specimen). 9. Metacercaria, showing reserve excretory system (drawn from a live specimen).

Discussion

The present form shows resemblance with *T. ranae* Kaw, 1950 ; *T. ujjainensis* Trivedi 1964 ; *T. muscularis* Chakrabarti 1970a ; *T. baughi* Pandey 1973 ; *T. tandoni* Pandey 1973 ; and *T. lymnaei* Pandey and Agrawal 1978 in having a divided body. It differs from *T. ranae* in presence of oesophagus from *T. ujjainensis* and *T. lymnaei* in the number of genital rudiment from *T. tandoni* and *T. baughi* in the relative size of suckers and number of genital rudiments and from *T. muscularis* in having a bilobed hold fast gland and poorly developed genital rudiments.

It resembles also with *Tetracotyle* of *A. pellucidus* Yamaguti, 1933 and *Tetracotyle* of *A. fuligulae* Yamaguti 1933 in having a divided body. However, it differs from *Tetracotyle* of *A. pellucidus* in the ratio of suckers and number of genital rudiments and from *Tetracotyle* of *A. fuligulae* in the ratio of suckers and absence of prepharynx.

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References

- Agrawal N 1975 A new strigeid larva (*Tetracotyle bufoi* n.sp.) from a common toad; *Indian J. Zool.* **16** 187-188
- Chakrabarti K K 1970a A double metacercarial infection in an Indian freshwater fish *Heteropneustes fossilis* (Bl.); *Rev. Biol. Trop.* **17** 91-96
- Chakrabarti K K 1970b Two new species of strigeid metacercaria from an Indian freshwater fish, *Xenentodon cancila* (Ham.); *Proc. Helm. Soc. Wash.* **37** 5-10
- Chakrabarti K K 1970c A new strigeid metacercaria, *Tetracotyle glossogobi* sp.n., from an Indian freshwater fish *Glossogobius giuris* (Ham.); *Helminthologia* **11** 1-4
- Chakrabarti K K and Baugh S C 1970 *Tetracotyle szidati* n.sp. a metacercaria from the Indian freshwater fish *Channa punctatus* (Bl.); *Indian J. Zool.* **11** 79-82
- Hughes R C 1928 Studies on the trematode family Strigeidae (Holostomidae) No. XIII. Three new species of *Tetracotyle*; *Trans. Am. Micr. Soc.* **47** 414-433
- Kaw B L 1950 Studies in helminthology. Helminth parasites of Kashmir. Part I. Trematode; *Indian J. Helminth.* **2** 67-126
- Pandey K C 1971a Studies on the trematode parasites of fishes of Lucknow (India)-I; *Proc. Nat. Acad. Sci. India* **41** 311-314
- Pandey K C 1971b Studies on metacercariae of freshwater fishes of India. VII. On morphology of *Tetracotyle lucknowensis* n.sp. from *Channa striatus* Bloch.; *Proc. Indian Acad. Sci. (Anim. Sci.)* **74** 1-5
- Pandey K C 1973 Studies on metacercariae of freshwater fishes of India. II. Description of a known and five unknown strigeid metacercariae from Lucknow; *Indian J. Zool.* **14** 155-166
- Pandey K C and Agrawal N 1978 A new *Tetracotyle* larva, *T. lymnaei* n.sp. from a freshwater mollusc *Lymnaea auricularia*; *Indian J. Parasit.* **2** 199-210
- Singh K S 1956 On some strigeid from India; *J. Zool. Soc. India* **8** 47-56
- Trivedi H S 1964 On two metacercariae from freshwater fishes in Ujjain; *J. Vsk. Univ.* **3** 91-94
- Yamaguti S 1933 Studies on the helminth fauna in Japan. Pt. I. Trematoda of birds, reptiles and mammals; *Jpn. J. Zool.* **5** 1-134

Toxic and sublethal effects of endosulfan on *Barbus stigma* (Pisces : Cyprinidae)*

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Abstract. Toxicity and the effect of sub-lethal concentrations of endosulfan on a fresh water fish *Barbus stigma* had been studied. Endosulfan proved to be lethal to *B. stigma* at a concentration of 0.01 ppm and above. The LC_{50} was 0.0043 ppm and the sub-lethal concentration was 0.003 ppm and below. At sub-lethal concentrations the fish exhibited erratic swimming activity and at lethal concentrations it lost the sense of balance. The rate of feeding was reduced by 5.94% to 9.02% and assimilation by 6.44 to 9.60% in different sub-lethal concentrations. Growth (weight) retarded from 11.6 mg/day in the control fish to 7.3, 6.0 and 5.1 mg/day in the endosulfan treated fishes. Respiratory rate of the pesticide treated fish also dropped by 10 to 16.6%. Due to the over all effect of the toxicity, the fish *B. stigma* comparatively showed a poor nutritive value by displaying a drop of nearly 35% in the organic constituents.

Keywords. Endosulfan ; pesticide ; lethal concentration ; sublethal concentrations

1. Introduction

Although pesticides produce good many results in the control of pests, their harmful effects on the non-target animals are also not ruled out. Pesticides leave residues in water and mud even several days after their spray in the adjacent crop fields. This poses a constant threat to the non target organisms, especially to the fishes, because pesticides are known to alter their behaviour pattern (Anderson 1971), growth and nutritional value (Korschgen and Murphy 1967; Arunachalam *et al* 1980; Yaganobano *et al* 1981), reproductive potential (Johnson 1967), cellular morphology (Mukhopadhyay and Dehadrai 1980) and Physiology (Baskaran 1980; Natarajan 1981). Though a good number of literatures are available on the toxicity of pesticides in fishes, studies on the sublethal concentrations of toxicants are meagre. The objective of the present study is to find out the effect of sublethal concentrations of endosulfan (the most effective and widely used pesticide in the field) on survival, behaviour, energy budget, respiratory pattern and the organic constituents of *B. stigma*.

* A part of the M.Phil. dissertation submitted by T. Manoharan to the Madurai Kamaraj University.

2. Materials and methods

The fish *B. stigma*, used in the present experiment, is edible, commercially valuable, and distributed all over India. The fishes were obtained from the Public Works Department and stocked in glass aquaria, after dipping in a 3.5% salt solution to prevent any parasitic attack. They were acclimatized to the laboratory condition for ten days and were fed on Oligochaete worm *Tubifex tubifex*. Preliminary tests were conducted in five aquaria containing five individuals each, to find out the toxicity range of the toxicant. The mortality range was assessed by using five arbitrarily chosen concentrations of endosulfan. For pesticide dilutions the static bioassay method (APHA 1971) was employed. After determining the mortality range (100% mortality) of the pesticide, desired concentrations down to the sublethal dose were prepared by diluting 35% EC endosulfan.

To find out the LC_{50} of 1.0 g unit weight of *B. stigma*, mortality rate was observed for 96 hrs at different arbitrarily chosen concentrations of endosulfan. At a concentration of 0.0043 ppm 50% of the fishes died at the end of 96 hrs. At 0.003 ppm and below, all fishes survived over a period of 30 days. Thus, 0.0043 ppm and 0.003 ppm of endosulfan were taken as LC_{50} and sublethal concentrations respectively. Following the method of Sprague (1973), the LC_{50} curve was drawn and mortality rate and concentration were expressed in probit and log values.

2.1. Experiments in sublethal concentrations

Experiments were conducted in three different concentrations (0.003, 0.002 and 0.001 ppm) of endosulfan. In each concentration three replicates and one control (without insecticide) were used simultaneously and the experiment was carried out for 20 days at $28 \pm 1^\circ \text{C}$. The fishes were fed on freshworms of *T. tubifex ad libitum* for 3 hrs/day. The unfed food was collected carefully by a pipette and the faeces by filtering the water daily. Water was changed once in a day. Both the left over food and the excreta were dried to constant weight at 90°C . Water content of the fish and the worms were determined by using the sacrifice method (Maynard and Loosli 1962). The scheme of energy balance was expressed by IBP formula (Petrusewicz and MacFadyen 1970), i.e.,

$$C = P + R + F + U$$

Where C = Food consumed ; P = Production (i.e. difference between the initial dry weight and the final dry weight) ; R = Respiration ; F = Faeces and U = Nitrogenous excretory products.

Assimilation was estimated by subtracting " F " from " C ". Assimilation efficiency was calculated as the percentage of food assimilated in relation to food consumed, gross (K_1) and net (K_2) conversion efficiencies were represented as percentage of food converted in relation to food consumed and assimilated respectively. Rates of feeding, assimilation and production were calculated to the respective quantities of food consumed, assimilated and converted relating to per unit live weight (g) of the fish per unit time (day).

2.1a. *Statistical analysis* : Different sublethal concentrations of endosulfan were correlated with rates of feeding, assimilation and conversion.

2.1b. *Specific growth rate*: Specific growth rate (mg/day) was calculated using the method adopted by Kosi Onodera (1962).

2.2. *Respiratory studies*

Control and experimental fishes were introduced into separate troughs containing two litres of water. A thin layer of Kerosene was layered carefully on the surface of the water to avoid the diffusion of atmospheric oxygen. After 30 min, 200 ml of water was siphoned out from each of the troughs and the oxygen content was estimated (Winkler 1948). The oxygen consumed by the experimental and control fishes were calculated by subtracting the value from the initial oxygen content of water.

2.3. *Bio-chemical analysis*

At the end of experiment (after 20 days), 5 mg of dried powder of total homogenate of control and experimental fishes were used and the total protein (Lowry *et al* 1951), the total lipid (Bragdon 1951) and the total sugar (Seifter *et al* 1950) were colorimetrically estimated.

3. Results

Endosulfan caused 100% mortality within 24 hrs of exposure at a concentration of 0.01 ppm (lethal). The LC_{50} (figure 1) was 0.0043 ppm during the 96 hrs of exposure. At the concentration of 0.003 ppm and below no mortality was observed (sublethal).

3.1. *Behaviour*

There was a marked increase in the swimming activity of the fishes immediately after they were transferred to lethal and sublethal concentrations.

3.2. *Feeding rate (table 1)*

Sublethal concentrations of endosulfan affected almost equally all the intermediary processes connected to food utilization (figure 2). The average feeding rate of test fish reared in fresh water (control) was 18.84 mg dry food/g live fish/day. This value decreased to 17.72 (5.9%), 17.52 (7%) and 17.14 (9.02%) mg dry food/g live fish/day, when they were reared in 0.001, 0.002 and 0.003 ppm concentration of endosulfan respectively.

3.3. *Assimilation rate (table 1)*

Assimilation rate also decreased from 17.07 mg dry food/g live fish/day (control) to 15.97 (6.44%), 15.89 (6.91%) and 15.43 (9.60%) in the experimental fishes (figure 3).

3.4. *Production rate (table 1)*

Fish growth was found to have retarded with increased concentrations of endosulfan in the medium (figure 4). The average production rate of *B. stigma* was

Table 1. Effects of different sublethal concentrations of Endosulfan on food utilization and efficiencies in *Barbus stigma*.

Parameters	Concentration			
	0.000	0.001 ppm	0.002 ppm	0.003 ppm
Feeding rate (Cr)	18.84±0.98	17.72±1.10	17.52±0.33	17.14±0.85
Assimilation rate (Ar)	17.07±0.65	15.97±0.98	15.89±0.99	15.43±0.54
Production rate (Pr)	2.47±0.17	1.41±0.40	1.23±0.33	1.14±0.50
Assimilation efficiency	90.61±0.74	90.07±0.39	90.65±0.51	89.76±0.31
Gross conversion efficiency	13.09±0.96	7.97±0.35	7.07±0.89	5.88±0.85
Net conversion efficiency	14.54±0.95	8.81±0.33	7.80±0.82	7.37±0.49

Each value represents the average performance of three (mean ± SD) individuals observed for 20 days at 28°C ± 1°C. Rates are expressed in mg dry weight/g live fish/day and the efficiencies are expressed in percentage.

2.47 mg dry substance/g live fish/day. The growth rate dropped to 1.41 (42.90%) in 0.001 ppm, 1.23 (50.20%) in 0.002 ppm and 1.14 (53.84%) in 0.003 ppm concentration of endosulfan.

Correlation coefficient values obtained between different sublethal concentrations of endosulfan and feeding ($r = -0.95$), assimilation ($r = -0.93$) and conversion ($r = -0.88$) rates were negatively correlated and the values were significant at 0.1% level.

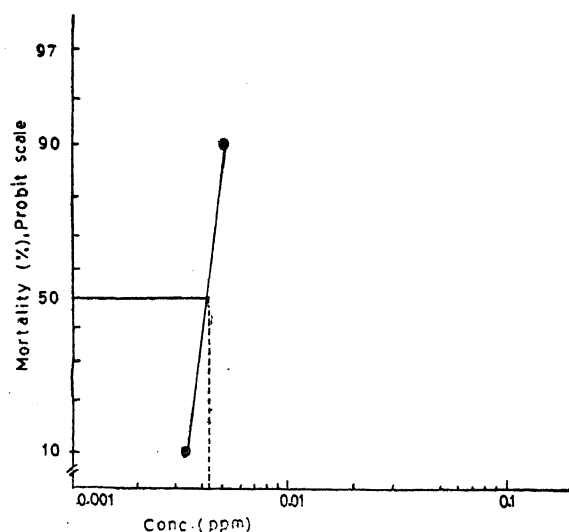


Figure 1. Dotted line indicates the LC₅₀ value at 96 hr exposure.

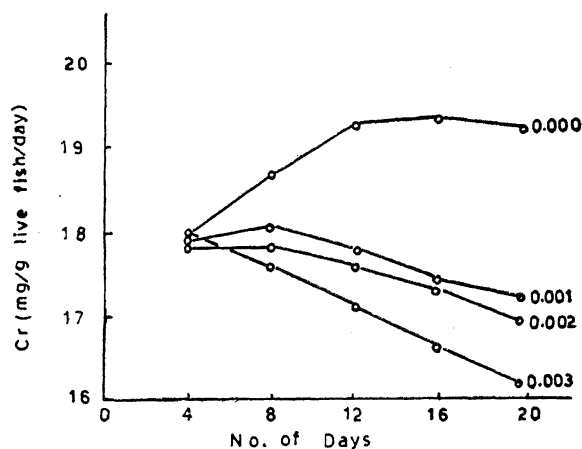


Figure 2. Effects of different sublethal concentrations of Endosulfan on the feeding rate of *B. stigma*.

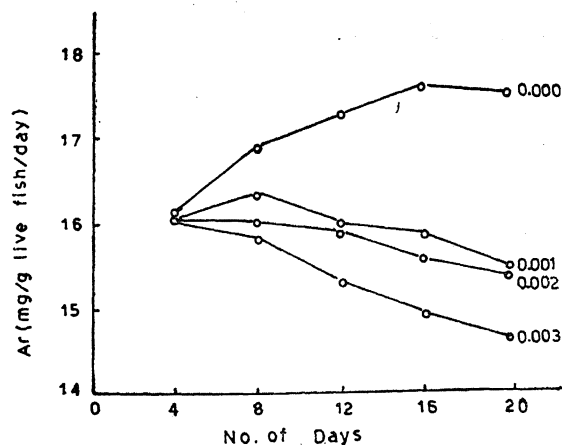


Figure 3. Effects of different sublethal concentrations of Endosulfan on the assimilation rate of *B. stigma*.

3.5. Efficiencies of Assimilation, gross (K_1) and net (K_2) conversion (table 1)

Endosulfan did not affect assimilation efficiency which averaged as 90%. The gross conversion efficiency (K_1) of the fish reared in fresh water was 13.09%, while the value was decreased in different sublethal concentrations (7.97%, 7.07% and 5.88% in 0.001, 0.002 and 0.003 ppm respectively). Net conversion efficiency (K_2) of the control fish was 14.54%, while the value was reduced to 8.81% in 0.001 ppm, 7.89% in 0.002 ppm and 7.39% in 0.003 ppm (figure 5).

3.5a. *Specific growth rate*: Specific growth rate of the control fish was 11.60 mg/day. This value decreased to 7.3, 6.0 and 5.1mg/day in 0.001, 0.002 and 0.003 ppm of endosulfan media respectively (figure 6).

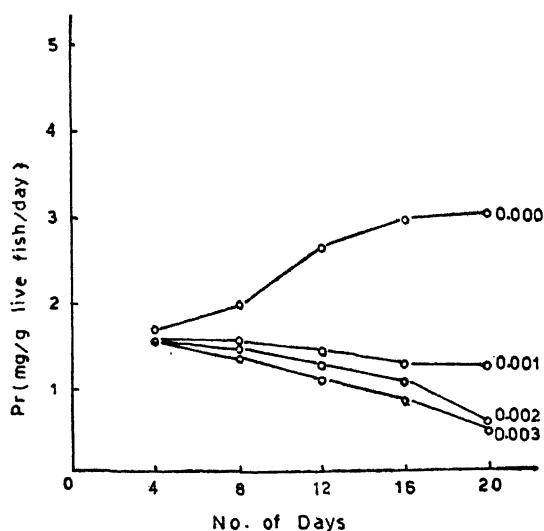


Figure 4. Effects of different sublethal concentrations of Endosulfan on the production rate of *B. stigma*.

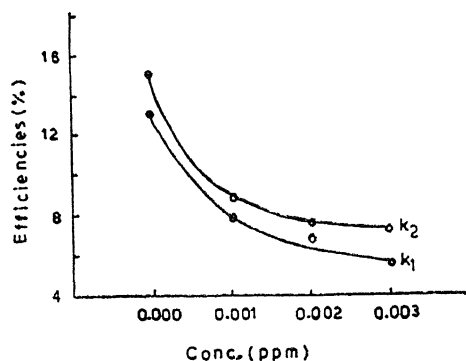


Figure 5. Effects of different sublethal concentrations of Endosulfan on the gross (K_1) and net (K_2) conversion efficiencies of *B. stigma*.

3.6. Respiration

The respiratory rate of the fish reared in pesticide-free water (control) was 1.08 mlo_2/g live fish/hr. In 0.001 ppm of endosulfan, the fish maintained more or less identical value. However, when the concentration was increased to 0.002 and 0.003 ppm the respiratory rate declined by 10.8% and 16.6%.

3.7. Organic constituents

The average values of protein, lipid and sugar content in the control fish were 280, 118.30 and 50.50 mg/g dry weight of the fish. The experimental fishes

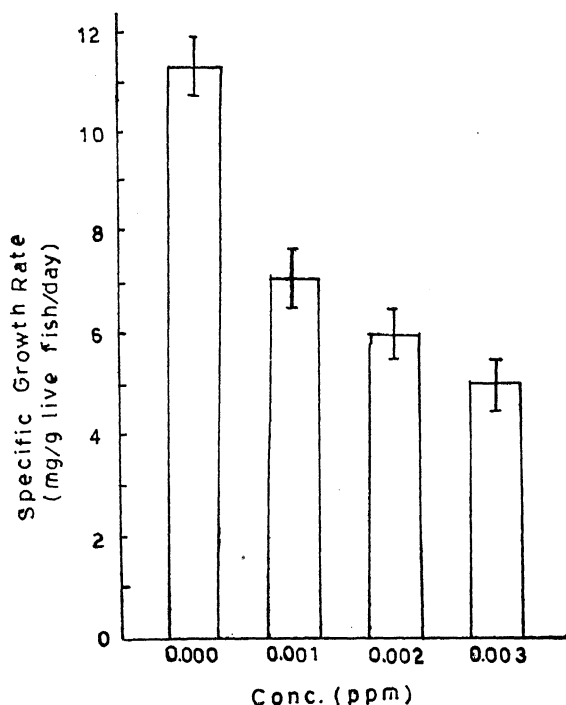


Figure 6. Effects of different sublethal concentrations of Endosulfan on the specific growth rate of *B. stigma*.

exhibited a significant drop in their organic constituents. Protein content was found to have dropped to 233, 221 and 180 mg/g in 0.001, 0.002 and 0.003 ppm endosulfan treated fishes. The value of lipid also declined from 118.30 to 97.56, 91.40 and 77.70 mg/g. Similarly sugar content of the experimental fishes also reduced from 50.50 to 45.00 in 0.001 ppm, 43.50 in 0.002 ppm and 40.00 in 0.003 ppm concentration.

4. Discussion

4.1 LC_{50} and sublethal concentration

The pesticide endosulfan has produced lethal effect at 0.01 ppm in *B. stigma*. The LC_{50} is 0.0043 ppm, at which 50% of fishes died within 96 hrs of exposure. Fishes survived at 0.003 ppm and below, indicating that 0.003 ppm is the sublethal level. In their toxicity studies, using endosulfan, Amminikutty and Rege (1971) observed that 0.0016 ppm is the LC_{50} for the fish *Gymnocorymbus ternetzi*. The data obtained in the present work indicates that different fishes have different tolerance range against the toxic effects of the same pesticide. In this way *B. stigma* seems to be more tolerant (about three times) to endosulfan than *G. ternetzi*.

4.1a. *Behaviour* : At sublethal concentrations the fish becomes restless, exhibiting erratic swimming activity and at lethal doses it loses its balance. A similar observation has been made in *Ictalurus punctatus* and *Channa punctatus* by Carter (1971) and Munawar Ahmed Anees (1975) respectively. Desi *et al* (1974) and Kingsley (1973), from their neurotoxicological studies, concluded that cholinesterase activity of various parts of nervous system is affected by the pesticide leading to the imbalance of the animal. In the present case also the disturbed swimming activity of the fish may be explained in the same line.

4.2. *Rates of feeding, assimilation and production*

Rate of feeding, assimilation and growth declined in *B. stigma* with increased concentrations of endosulfan in the medium. This observation falls in line with the findings made by Arunachalam *et al* (1980) and Baskaran (1980), in *Mystus vittatus* and *Channa striatus*. The decrease in growth and conversion efficiency may be due to the diversion of more energy for the stress put up against the toxic effect of the pesticide, as suggested by Arunachalam *et al* (1980).

4.3. *Respiration*

Respiratory rate decreases up to 16.7% in endosulfan treated fishes. This result is in conformation with the earlier reports by Baskaran (1980) in *C. striatus* using DDT and methyl parathion and by Carolyn *et al* (1976) using Carbaryl and Dieldrin on Rainbow trout. Blood smear studies made in the present experiments have revealed the severe damage caused to the red blood corpuscles in the endosulfan treated fishes. A reduction in the RBC count in *C. striatus* following exposure to Metasystox (Demeton) has been reported by Natarajan (1981). Therefore, it is presumed that due to the injury caused to the red blood corpuscles by the pesticides, the efficiency of the fish to trap the dissolved oxygen is considerably reduced resulting in the reduced rate of respiration.

4.4. *Nutritive value of the fish*

Finally the data obtained in the present experiments have shown that the nutritive value of the fish treated with pesticide is significantly reduced (16 to 35% drop in protein, 17 to 34% in lipid and 9 to 20% drop in sugar level). A similar phenomenon has been demonstrated by Ramana Rao and Ramamurthi (1980) and Kabeer Ahmed *et al* (1978) in Sumithion treated *Pila globosa* and Malathion treated *Lamellidens marginalis*. Although the above references are from invertebrates, they prompt one to think that such a phenomenon (reduced level of organic constituents) is common to all animals irrespective of species.

5. *Conclusion*

From this investigation it is obvious that the toxic nature of pesticides produces lethal effect in fishes at higher doses. Even in sublethal concentrations, it results

in degraded metabolic changes, affecting the nutritive value of the animal. Therefore it may be suggested that necessary care may be taken to avoid contamination of fresh water bodies while spraying pesticides.

Acknowledgement

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References

- Amminikutty C K and Rege M S 1977 Effects of acute and chronic exposure to pesticides, Thiodan BC 35 and Agallol '3' on the liver of Widowtetra *Cymnecorymbus ternetzi*; *Indian J. Expl. Biol.* 15 197-200
- Anderson J M 1971 Sublethal effects and changes in ecosystems. Assessment of the pollutants on physiology and behaviour; *Proc. R.Soc. London* 177 307-320
- APHA 1971 American Public Health Association New York 1971 *In Standard methods for the examination of water and sewage* 13th edition.
- Arunachalam S, Jeyalakshmi K and Aboobucker S 1980 Toxic and sublethal effects of Carbaryl on a fresh water catfish *Mystus vittatus*; *Arch. Environ. Contam. Toxicol.* 9 307-316
- Baskaran R 1980 Biological studies on a chosen thermo conformer (*Channa striatus*); Ph.D. thesis submitted to Madurai Kamaraj University, Madurai
- Bragdon J H 1951 Colorimetric determination of blood lipids; *J. Biol. Chem.* 190 513
- Carolyn R Lunn, Daniel P Toews and David J Pree 1976 Effects of three pesticides on respiration, coughing and heart rates of Rainbow trout; *Can. J. Zool.* 54 214-219
- Carter F L 1971 *In vitro* studies of brain cholinesterase inhibition by organo phosphate and carbamate insecticides in fish; Ph.D. thesis, University of Louisiana, Baton rouge, Louisiana (Diss. Abstr. Int.) 13 2772-73
- Desi Ilees, Lili Genczi Gyorgy Simon, Ildiko Farkas and Zsuzsa Kneffel 1974 Neurotoxicological studies of two carbamate pesticides in sub-acute animal experiments; *Toxicol. Appl. Pharmacol.* 27 465
- *Johnson H E 1967 The effects of Endrin in the reproduction of a freshwater fish, *Cryzias latipes*; Ph.D. thesis, Univ. Washington, Seattle, Wash.
- Kabeer Ahamed I, Begum Md R, Sivaiah S and Ramana Rao K V 1978 Effect of Malathion on free amino acids, total proteins, glycogen and some enzymes of Pelecypod *Lamellidens marginalis*; *Proc. Indian Acad. Sci. (Anim. Sci.)* 87 377-380
- Kingsley Kay 1973 Toxicity of pesticides; Recent advances, *Environ. Res.* 6 202
- Korschgen L J and Murphy D A 1967 Missouri federal aid project progress report
- Kosi Onodera 1962 Some data on Eel culture in Japan; *Indo-Pac. Fish. Council., Occasional Pap.* 6216 4
- Lowry O H, Rosebrough N J, Farr A L and Randal R J 1951 Protein measurement with Folin phenol reagent; *J. Biol. Chem.* 193 265-275
- Maynard A L and Loosli K J 1962 *Animal nutrition* 5th ed. (New York: McGraw-Hill)
- Mukhopadhyay R K and Dehadrai P V 1980 Studies on air breathing catfish *Clarias batrachus* under Malathion sublethal exposure; *Indian J. Exp. Biol.* 18 348-352
- Munawar Ahmed Anees 1975 Acute toxicity of our organophosphorous insecticides to a fresh water teleost *Channa punctatus*; *Pakistan J. Zool.* 7 135
- Natarajan G M 1981 Changes in the bimodel gas exchange and some blood parameters in the air breathing fish, *Channa striatus* following lethal (LC_{50/48} hrs) exposure to Metasystox (Demeton); *Current Sci.* 50 40-41
- Petrusewicz and MacFadyen A 1970 *Productivity of terrestrial animals*; IBP handbook No. 13 (Oxford: Blackwell)
- Ramanarao M V and Ramamurthi R 1980 Effect of sublethal concentration of sumithion on some biochemical constituents of the freshwater snail *Pila globosa* *Geobios* 7 247-250

- Seifter S and Dayton S 1950 The estimation of glycogen with anthrone reagent ; *Arch. Biochem. Biophys.* **25** 191-200
- Sprague J B 1973 *The ABC's of pollutant Bioassay using Fish* ; American Society for Testing and Materials 6-30
- Winkler 1948 In Welch P S *Limnological methods* (New York : McGraw-Hill)
- Yaganobano, Seikh Amjad Ali and Tariq Hameed 1981 Effects of sublethal concentration of DDT on muscle constituents of an air breathing catfish, *Clarias batrachus* ; *Proc. Indian Acad. Sci. (Anim. Sci.)*, **90** 33-37

* Originals not referred.

Interruption of pregnancy by barbiturates in albino rats

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Abstract. Barbiturates inhibit the LH surge and release of gonadotrophins (LH and FSH) and prolactin from the pituitary in rats and hamsters. In the present study administration of phenobarbital (7.5 mg) or barbital sodium (20 mg) twice a day from day 8-11 interrupts the pregnancy in rats with little or no foetal survival. The ovaries and the uterus of these rats resemble those of non-pregnant rats when autopsied on day 20 of pregnancy. These results suggest that the failure of maintenance of pregnancy by barbiturate treatment may be due to the inhibition of luteotrophic hormones from the pituitary during the crucial period of pregnancy, resulting in the insufficient secretion and release of ovarian progesterone and also estrogen.

Keywords. Gonadotrophins; pituitary; phenobarbital; barbital sodium; luteotrophins; corpora lutea; fetuses.

1. Introduction

Maintenance of pregnancy is the consorted efforts of all endocrine glands mediated through the hypothalamo-hypophyseal-ovarian and placental axes. Hypophysis is indispensable during first half of pregnancy, but the ovaries are essential throughout the gestation, as the placental gonadotrophins take over the functions of the pituitary gonadotrophins during later part of pregnancy in rats (Lyons and Ahmad 1973; Rothchild *et al* 1974). Maintenance of pregnancy by exogenous LH or other luteotrophins in hypophysectomized rats, and by proper doses of progesterone and estrogens in ovariectomized rats and hamsters is achieved by several investigators (Jagannadha Rao *et al* 1972; Yoshinaga *et al* 1972).

Studies with pheno- and pentobarbital indicate that these drugs inhibit the pituitary LH surge and tonic release of FSH, LH and prolactin, and also interfere with the ovarian steroidogenesis directly (Gupta and Karavolas 1973; Norman *et al* 1973; Blake 1974). As barbiturates interfere with much of the endocrine activities of pituitary and ovary that are essential for the maintenance of pregnancy, the aim of the present investigation was to study the effects of phenobarbital and barbital sodium on pregnancy in rats.

2. Material and methods

Normal cycling, nulliparous rats of Holtzman strain, weighing 140–150 g, 80–90 days old were mated with fertile males at proestrus or early estrus. The rats showing spermatozoa in the vaginal smears on the subsequent day were selected for experimentation and that day was designated as day 1 of pregnancy. The selected rats were laparotomized under mild ether anaesthesia on day 8 of pregnancy to note the number of implantations and those having normal implantations were taken for the further experimentation.

2.1. Experiment I

To study the different dose effect of phenobarbital; 2.5 mg, 5.0 mg or 7.5 mg, phenobarbital/100 g body weight, in 0.5 ml saline was injected subcutaneously twice a day from day 8 through day 19 of pregnancy.

2.2. Experiment II

To study the different dose effect of barbital sodium 10 mg, 15 mg or 20 mg barbital sodium/100 g body weight in 0.5 ml saline was injected subcutaneously, twice a day from day 8 through day 19 of pregnancy.

For the above experiments, saline treated controls were maintained. The drug treatment was continued until profuse vaginal bleeding was observed. All the rats were autopsied on day 20. The number of foetuses, placentomas, placental scars and placentae were recorded. Ovaries were weighed, fixed in Bouin's fluid, embedded in paraffin, sectioned and stained in haematoxylin-eosin for histological observations. The rats were maintained in individual cages with Hindustan Lever rat feed and water *ad libitum* at a room temperature of $27 \pm 1^\circ\text{C}$ with 12 hrs of lighting schedule.

3. Results

3.1. Interruption of pregnancy (tables 1 and 2)

Administration of different doses of phenobarbital or barbital sodium, twice a day from day 8 through day 19 interrupts the pregnancy in rats to various levels. Low doses of phenobarbital i.e. 2.5 mg/100 g body weight interrupts pregnancy in 1/5 rats, while 5 mg of the same drug causes partial maintenance in 2/5 rats and the remaining rats in these groups exhibit successful maintenance of pregnancy to full term.

Similarly 10 or 15 mg barbital sodium is not effective in interrupting the pregnancy wherein 5/6 rats or 5/5 rats maintain the pregnancy completely up to day 20. Only one rat with 10 mg barbital sodium exhibits partial maintenance.

These results indicate that the phenobarbital is more potent than barbital sodium in affecting the pregnancy even in low doses. The effective dose of phenobarbital or barbital sodium in interrupting the pregnancy is 7.5 mg or 20 mg respectively wherein 9/9 rats or 8/9 rats show complete abortion with profuse vaginal bleeding on day 12 or 13 of pregnancy. In saline treated controls, the pregnancy is maintained successfully in almost all rats.

Table 1. Effect of graded doses of phenobarbital on pregnancy in rats.

Treatment—day 8-19

Dose/100 gms body wt. 2 doses/day

Treatment	Mean in relation to pregnant at laparotomy M \pm S.E.				% foetal survival	Ovarian wt. mg/ 100 gms body wt. M \pm S.E.
	Implan- tations	Placen- tal scars	Placentomas	Live foet uses		
Control	7.60	0.2	...	7.40	97.4	39.89
(5)	\pm	\pm		\pm		\pm
	1.51	0.2		1.51		4.19
Phenobarbital	7.40	1.40	...	6.20	83.8	40.61
2.5 mg	\pm	\pm		\pm		\pm
(5)	0.67	1.39		1.70		7.75
5.0 mg	8.60	0.8	...	7.60	88.4	40.23
(5)	\pm	\pm		\pm		\pm
	0.51	0.37		0.56		1.37
7.5 mg	7.50	0.00	31.17*
(9)	\pm					\pm
	0.59					1.68

Laparotomy is done on day 8 and autopsy on day 20 of pregnancy.

Number in paranthesis denotes the number of rats.

M \pm S.E. = Mean \pm Std. error. * P < 0.05.

3.2. Foetal survival

The percent foetal survival is calculated in relation to number of live foetuses on day 20, with reference to the number of implantations observed on day 8, at laparotomy. In saline treated controls 37 foetuses were found out of 38 implantations indicating 97.4% foetal survival. With 2.5 mg or 5.0 mg phenobarbital treatment 83.8% or 88.4% respective foetal survival is observed. But with 10 mg or 15mg barbital sodium administration the respective foetal survival is 97.9 or 100.0% which is almost similar compared to that of controls. However, 7.5 mg phenobarbital or 20 mg barbital sodium administration, the implantation loss is considerable, wherein the number of foetuses vs implantation sites is 0/60 or 7/63 respectively, thereby indicating that foetal survival is nil or 11.1% with respective to drug treatment.

The above results indicate that phenobarbital is more potent in its litter destroying effect than barbital sodium, which may be due to its prolonged action on the central nervous system.

Table 2. Effect of graded doses of barbital sodium on pregnancy in rats.

Treatment-day 8-19

Dose/100 gms body wt. 2 doses/day

Treatment	Mean in relation to pregnant at laparotomy M \pm S.E.				% foetal survival	Ovarian wt. mg/ 100 gms body wt. M \pm S.E.
	Implan- tations	Placental scars	Placen- tomas	Live foetuses		
Control	7.60	0.2	...	7.40	97.4	39.89
(5)	\pm	\pm		\pm		\pm
	1.51	0.2		1.51		4.19
Barbital sodium	7.83	0.17	...	7.66	97.9	36.60
10 mg	\pm	\pm		\pm		\pm
(6)	0.48	0.17		0.37		1.50
15 mg	8.20	8.20	100.0	42.2
(5)	\pm			\pm		\pm
	0.36			0.36		3.66
20 mg	7.00	0.78	11.1	25.59**
(10)	\pm			\pm		\pm
	0.41			0.78		1.46

Laparotomy is done on day 8 and autopsy on day 20 of pregnancy.

Number in paranthesis denotes the number of rats.

M \pm S.E. = Mean \pm Std. error. ** $P < 0.01$.

3.3. Gravimetric and histological changes of the ovary

In the controls, where the pregnancy is maintained to full term, the ovaries are large with well developed corpora lutea, weighing 38.89 mg. With 2.5 mg or 5.0 mg phenobarbital treatment, wherein pregnancy is not much affected, the ovary exhibits large well developed corpora lutea similar to those of controls. However in the rats treated with 7.5 mg phenobarbital or 20 mg barbital sodium, where the complete abortion has occurred, with almost nil foetal survival, the ovaries are small with moderate sized corpora lutea and ovulated follicles. The ovaries are reduced significantly weighing 37.17 mg ($P < 0.05$) or 25.59 mg ($P < 0.01$) with the administration of phenobarbital or barbital sodium respectively. These aborted rats come to estrus within 3-4 days after profuse vaginal bleeding and hence the ovaries resemble those of nonpregnant rats. These results indicate a good correlation between the percent foetal survival, ovarian weight and its histology.

The adverse effect of barbiturates on pregnancy seems to be due to blockade of pituitary gonadotrophins release during the critical period of gestation (day 8-11),

wherein the pituitary hormone balance is essential for the normal functioning of the ovaries which are responsible for the maintenance of gestation during its first half.

4. Discussion

The apparent neutralization of LH during days 7-11 of pregnancy in rats results in the termination of gestation by foetal resorption (Rothchild *et al* 1974). Initiation of a rise in the progesterone synthesis and pituitary LH release coincides with an increased follicular growth and hypertrophy of corpora lutea between day 9-12 of pregnancy in rats and hamsters (Greenwald 1973 ; Rothchild *et al* 1974). Therefore it is evident that pituitary LH is essential for the maintenance of corpora lutea in the functional state as to produce progesterone, sufficient to maintain the pregnancy during the early half. In the present investigation, phenobarbital (7.5 mg) or barbital sodium (20 mg) causes profuse vaginal bleeding with foetal loss in 8/8 or 8/9 rats respectively when treated from day 8-11. The ovaries of these rats are significantly reduced with very small corpora lutea and resemble to those of non-pregnant rats when observed after autopsy on day 20. The probable *modus operandi* is the continued inhibition of pituitary LH release during the crucial period of pregnancy by the chronic treatment of barbiturates, as these drugs are known to inhibit the LH surge and release in rats and hamsters (Norman *et al* 1973 ; Blake 1974 ; McCormack 1974). Therefore for all probabilities, the ovaries of barbiturate treated rats may not be functional due to LH inhibition, as LH stimulates the production of progesterone from corpus luteum, and Yoshinaga *et al* (1972) and Jagannadha Rao *et al* (1972) have observed a decrease in the progesterone and $20\alpha\text{—OH—P}$ by neutralizing endogenous LH by LH antiserum treatment. Therefore the corpora lutea of pregnant rats seem to be dependent upon LH to maintain the high progesterone levels during gestation. It can be postulated that interruption of pregnancy by chronic treatment of barbiturates is due to continued blockade or lowering of LH, resulting in subnormal production of progesterone. Besides, it has been reported that barbiturates interfere directly with the ovarian steroidogenesis by decreasing the 3β -hydroxy steroid dehydrogenase activities (Gupta and Karavolas 1973).

It is also stated by Greenwald and co-workers (1973, 1974) that prolactin with FSH or estrone forms the luteotrophic complex during the early part of pregnancy. These luteotrophins might have been decreased in barbiturate treated rats, as barbiturates inhibit both gonadotrophins (FSH and LH) and prolactin release (Ajika *et al* 1972 ; Beatti *et al* 1973). The ineffectiveness of low doses of these drugs causing abortion or foetal resorption may be due to their failure in inhibiting the pituitary gonadotrophins and prolactin effectively. Therefore it can be concluded that the interruption of pregnancy in barbiturate treated rats is not only because of the decreased pituitary gonadotrophins and prolactin release during the crucial period, but also due to the direct interference of these drugs in the ovarian steroidogenesis.

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References

- Ajika K, Kalra S P, Fawcett C P, Krulich I and McCann S M 1972 The effect of stress and nembutal on plasma levels of gonadotrophins and prolactin in ovariectomized rats; *Endocrinology* **90** 707-715
- Beattie C W, Campbell C S, Nequin L Q, Soyker L F and Schwartz N B 1973 Barbiturate blockade of tonic LH secretion in the male and female rats; *Endocrinology* **92** 1634-1638
- Blake C A 1974 Localization of the inhibitory action of the ovulation blocking drugs on release of luteinizing hormone in ovariectomized rat; *Endocrinology* **95** 999-1004
- Greenwald G S 1974 Modification in ovarian and pituitary function in the hypophysectomized pregnant hamster; *J. Endocrinol.* **61** 35-51
- Greenwald G S 1973 Further evidence for a luteotrophic complex in hamster; Progesterone determination of plasma and corpora lutea; *Endocrinology* **92** 235-242
- Gupta C and Karavolas R J 1973 Lowered ovarian conversion of 14 C-pregnenolone and other metabolites during phenobarbital (PB) block of PMS-induced ovulation in immature rats; inhibition of 3 β -hydroxy steroid dehydrogenase; *Endocrinology* **92** 117-124
- Jagannadha Rao A, Madhwa Raj H G and Moudugal N R 1972 Effect of LH, FSH and their antisera on gestation in hamster; (*Mesocricetus auratus*); *J. Reprod. Fert.* **29** 239-249
- Lyons W R and Ahmad N 1973 Hormonal maintenance of pregnancy in hypophysectomized rats; *Proc. Soc. Exp. Biol. Med.* **142** 198-202
- McCormack C E 1974 Reversal by progesterone of barbiturate blockade of ovulations: effect of concentration of serum LH (38098); *Proc. Soc. Exp. Biol. Med.* **146** 329-332
- Norman R L, Blake C A and Sawyer C H 1973 Evidence for neural sites, of action of phenobarbital and progesterone on LH release in the hamster; *Biol. Reprod.* **8** 83-86
- Rothchild I, Pepe G J and Morishige W K 1974 Factors affecting the dependency on LH in the regulation of corpus luteum progesterone secretion in the rat; *Endocrinology* **95** 280-288
- Yoshinaga K, McDonald G J and Greep R O 1972 Influence of various doses of LH on foetal survival in hypophysectomized rats; *Proc. Soc. Exp. Biol. Med.* **140** 193-195

Observations on the natural history and population ecology of the social wasp *Ropalidia marginata* (Lep.) from Peninsular India (Hymenoptera: Vespidae)

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Abstract. *Ropalidia marginata*, the most common Indian social wasp, belongs to a crucial stage of social evolution showing no obvious morphological caste differentiation but a behavioural caste differentiation and a dominance hierarchy that appears to influence division of labour. The nests consist of a single open comb that can sometimes have up to 500 cells and 10 pedicels. Nests are initiated and abandoned all round the year. Initiation is by 1-20 foundresses, 1-4 being the most common number. There is a great deal of variation in brood developmental times both within and between nests. Male progeny disappear from the nest soon after emergence while daughters stay on at the parent nest for a mean period of about a month. Small nests have a single egg layer while large nests have two or more females with well developed ovaries that presumably lay eggs. Most nests are short-lived, small nests being highly susceptible to failure. Large nests are less susceptible to failure but the emergence of multiple egg layers reduces the average relatedness of workers to the brood which presumably is the cause for large scale emigrations from these nests. An interaction of ecological and social factors therefore appears to determine the growth of a nest.

Keywords. Social wasp ; *Ropalidia marginata* ; natural history ; population ecology ; hymenoptera ; caste differentiation.

1. Introduction

Recent years have witnessed a great surge of interest in social hymenoptera because the emergence of a considerable body of theoretical ideas (Hamilton 1964 a,b ; Lin and Michener 1972 ; Alexander 1974) have raised hopes that herein lies the key to understanding the evolution of social behaviour (West-Eberhard 1969, 1975 ; Wilson 1971, 1975 ; Jeanne 1972, 1980 ; Michener 1974 ; Trivers and Hare 1976 ; Litter 1977, 1979, 1981 ; Starr 1979). Bees and wasps are of special interest in this connection because they exemplify a series of stages in the evolution of

sociality from the completely solitary to the highly advanced eusocial species (see Evans and West-Eberhard 1970 ; Michener 1974 ; Wilson 1971).

Ropalidia marginata is the commonest social wasp of Peninsular India (Van der Vecht 1962). This species shows cooperative brood care, reproductive caste differentiation and overlap of generations (Gadgil and Mahabal 1974 ; Gadagkar 1980 ; Gadagkar and Joshi 1982b, 1983a ; Gadagkar unpublished observations) and hence can be called *eusocial* according to the classification of Michener (1969). There is no obvious morphological differentiation between egg layers and non egg layers (Gadgil and Mahabal 1974) and division of labour is brought about by a dominance hierarchy among the females belonging to a nest (Gadagkar 1980). Analysis of the time-activity budgets of adults on *R. marginata* nests has in fact revealed the presence of a behavioural caste differentiation in this primitively eusocial wasp (Gadagkar and Joshi 1982b, 1983a).

Apart from these few recent studies there is very little information in the literature about this interesting genus (Roubaud 1916 ; Carl 1934 ; Darchen 1976 ; Belvadi and Govindan 1981 ; Gadagkar and Joshi 1982a,c, 1983b). Moreover, in addition to understanding reproductive differentiation and social organization, information on the dynamics of initiation, growth and extinction of colonies is essential before we even begin to speculate about the factors that might be responsible for the origin and maintenance of sociality. We present in this paper the results of our observations on the natural history as well as population ecology of *Ropalidia marginata* in Peninsular India.

2. Materials and methods

2.1. Study sites

In all we have observed 125 nests of *Ropalidia marginata* from the cities of Pune (18° 30' N and 73° 53' E) (45 nests) and Bangalore (13° 00' N and 77° 32' E) (80 nests) at various times over a period of nine years from October 1971 to October 1980.

2.2. Population fluctuations

Our population observations include records of the numbers of pupae and adults in a nest maintained at roughly 8–10 day intervals. Such observations were maintained on three nests in Pune from October 1971 to May 1973 and for 35 nests in Bangalore from October 1974 to October 1976. The 35 nests in Bangalore were all located on the windows of one building about 20,000 sq.ft. in area and a height of about 40 ft. Our records of the population in this site also provide information on (i) seasonal variations in numbers of adult wasps, pupae and nests, (ii) seasonality of initiation and abandoning of nests and (iii) life spans of nests.

2.3. Brood developmental times

For one nest in Pune and two nests in Bangalore the contents of each cell in the nest were noted to provide estimates of developmental times of the eggs, larvae and pupae.

2.4. Period of residence of adults on nests

Every adult on two nests in Bangalore was marked with a unique spot of quick drying paint immediately upon emergence without removing it from the nest. A census of all the adults present on the nests was taken on alternate days from November 1979 to June 1980 to provide records of the total period of residence of 60 females and 3 males.

2.5. Collection of nests

28 nests in Pune and 31 nests in Bangalore were collected taking precaution not to bias the sampling in favour of any particular size class of nests and to collect entire combs along with all the adults and immature stages. The numbers of pedicels, cells, eggs, larvae, pupae and adults were determined. The adults were sexed and the females were dissected to determine the state of development of their ovaries. The females were classified arbitrarily into 3 categories: those with undeveloped ovaries, those with moderately developed ovaries and those with well developed ovaries on the basis of maximum ovariole width. Those classified as 'with well-developed ovaries' appeared to have mature eggs and were probably laying eggs. These females are designated as egg layers. We do not however know if all females with well-developed ovaries actually laid eggs.

3. Results

3.1. The nest and its structure

R. marginata builds nests with simple, open (Gymnodomous according to the classification of de Saussure (1853-59) and Richards and Richards (1951)) combs the construction of which begins with the laying down of the pedicel which is usually 5-10 mm long and about 1 mm thick. The first cell is constructed at the tip of this pedicel and the subsequent cells are added either all round the first cell or only on one side so that in larger combs the initial pedicel may either end up being approximately in the centre of a layer of cells or at one extreme end. As the comb grows in size the initial pedicel is enlarged in width and may grow up to about 5-6 mm in diameter in large combs. In addition to enlarging the original pedicel, new thin pedicels (about 1 mm in diameter) that reinforce the attachment of the comb to the substratum are added at several points. Most small combs (< 100 cells) have a single pedicel while large combs (> 100 cells) often have more than one pedicel (table 1). The largest comb we have recorded had about 500 cells and 10 pedicels, the latter also being the largest number of pedicels recorded on a comb.

All but one of the nests recorded, has a single comb per nest. In one case however, there were two combs within about 20 mm of each other and the adults clearly moved between these two combs.

3.2. Initiation of nests

Nests of *R. marginata* are initiated and abandoned all round the year (table 2). New nests are initiated by 1-20 females, 1-9 being the commonest number (figure 1).

Table 1. Nests with different number of pedicels

Number of cells in nest	Frequency of nests with different number of pedicels					
	1 Pedicel	2 Pedicels	3 Pedicels	4 Pedicels	7 Pedicels	10 Pedicels
1-100	17	3
101-200	1	...	2
201-300
301-400	1	1	...	1
401-500	...	1	2	...	1	1

Table 2. Year-round initiation and abandoning of nests*

Month	Number of nests initiated	Number of nests abandoned
January	4	3
February	0	3
March	0	1
April	3	2
May	5	2
June	1	8
July	1	2
August	8	7
September	3	1
October	2	1
November	4	2
December	0	0

Data pooled from observations throughout the study period both in Bangalore and Pune.

In many cases the initial single foundress appears to be joined by other females within a few days of initiation of the nest. When newly emerging females were marked with spots of paint, it was noticed that some of the newly emerged individuals were not spending every night on the parent nest but were occasionally missing for 2-3 days before returning to it. It is possible that these individuals had been visiting other newly founded nests on the nights they were absent. There were emigrations of large number of adults from nests which had grown to more than 40-50 adults in size. Groups of individuals from these exoduses probably constitute the initial set of foundresses for many nests,

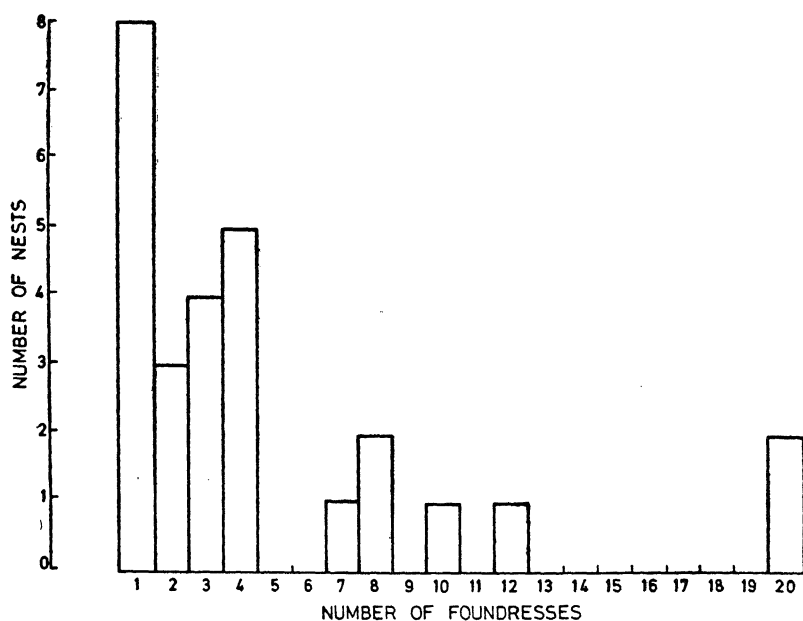


Figure 1. Frequency distribution of the number of nests with different numbers of foundresses.

3.3. Brood developmental times

The accurate determination of brood developmental times is beset with a number of problems and the estimates given here are only to be treated as first approximations. The duration of the egg, larval and pupal stages both in Pune and Bangalore are given in table 3. In each stage the duration in Pune is much less than in Bangalore. This difference could either be a genuine difference due to different environmental conditions in Pune and Bangalore. However, it cannot be ruled out that the differences are simply a result of small sample sizes in terms of the number of nests studied. The data in Pune in fact represent a single nest and that in Bangalore two nests. The difference could therefore be simply a manifestation of different stages in the nest cycle or of different local conditions. The nest in Pune, may have been located close to a good food source and therefore the difference may not even reflect differences between Pune and Bangalore as such.

The data both from Pune and Bangalore show a very great degree of variation. The standard deviations are close to half or sometimes more than half of the mean. The wide variation in egg developmental times is primarily because there is a significant degree of egg cannibalism which remains undetected. Eggs are eaten and replaced by new ones and several consecutive replacements may occur before an egg successfully hatches into a larva. The variation in larval developmental times almost certainly reflects differences in food supply. A larva can complete development and pupate in as little as 7 days in Bangalore under laboratory conditions when the adults feeding the larva are provided with an *ad libitum* food supply (Gadagkar, unpublished observations). The variations in pupal develop-

Table 3. Brood developmental times*

	From weekly observations in Pune			From daily observations in Pune			From weekly observation in Bangalore		
	Mean	Standard deviation	Sample size	Mean	Standard deviation	Sample size	Mean	Standard deviation	Sample size
Egg	18	13	1221	12	8	64	27	15	43
Larva	15	10	1052	10	5	37	22	7	28
Pupa	16	8	1071	14	6	45	29	11	16

* All means and standard deviations are in days.

mental times are the hardest to understand. The hypothesis that a strong correlation between larval and pupal developmental times is the cause of this variation is not borne out because we find that the correlation coefficients between larval and pupal developmental times are not significantly different from zero at 5% level. This is true even in the large sample size from weekly observations in Pune.

3.4. Duration of residence of adults on the nest

In all nests in which the newly emerging adults were marked it was observed that the males always disappeared within two to four days of emergence. While some females disappeared very soon after emergence, others stayed on at the parent nest for long periods of time. On two nests all the emerging adults (a total of 75 females and 3 males) were marked. The 3 males disappeared from the nest within 2, 3 and 4 days respectively of emergence. Of the 75 females we have information on the duration of residence on the nest for 60 females that disappeared before the end of our study. The frequency distribution of residence times for these 60 females is shown in figure 2. This corresponds to a (mean \pm SD) residence time of 27 ± 23 days and a range from 1-160 days. When a wasp disappears from one nest it may either have died (mortality) or initiated or joined another nest (emigration). In our records these two components cannot be distinguished directly. The (mean \pm SD) age-specific day to day probability of remaining at the same nest (inset, figure 3) has a value of 0.95 ± 0.04 which is nearly constant with age. This seems to suggest that mortality as opposed to emigration forms a very large component of our estimates. The reasoning behind this is that mortality seems to occur during the foraging trips because the wasps simply do not return to the nest at the end of the day. Perhaps they are lost or preyed upon. It is reasonable to assume that the probability of these events would be

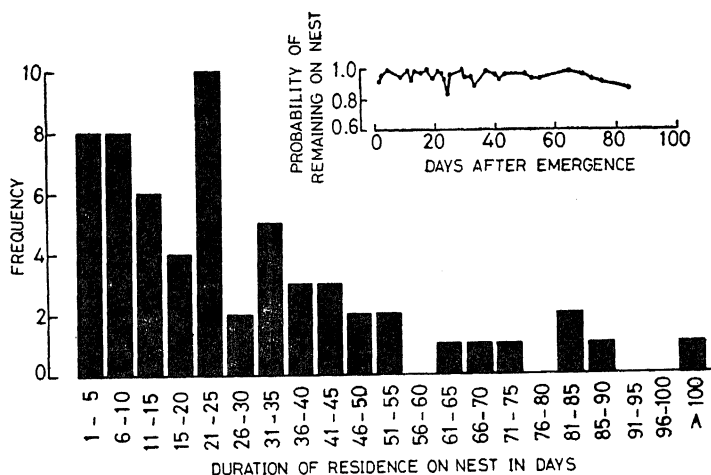


Figure 2. Frequency distribution of residence times on a given nest of 60 female wasps of *R. marginata*. The age-specific day-to-day probability of remaining at the same nest (inset) of 0.95 ± 0.04 is nearly constant with age. Note that most of the points lie between 0.90 and 1.0.

independent of the age of the animal but that the probability of emigration to found or join another nest would show some age dependence.

3.5. Reproductive differentiation

Our dissections from the harvested colonies indicate that although there is no morphological differentiation amongst the females, there is a marked differentiation amongst them in terms of development of ovaries. In all the nests, a majority of the females possessed rudimentary, completely undeveloped ovaries, while only 1 to 6 females possessed moderately or well developed ovaries. In most cases, the females with well-developed ovaries tended to be heavier in weight than the other females (Gadgil and Mahabal 1974). In addition there is a dominance hierarchy amongst the females at a nest with the dominant females doing less foraging (Gadagkar 1980). There is extensive food sharing at the nests of *R. marginata*, and since frequency of dominance behaviour and snatching food are significantly correlated (Gadagkar and Joshi 1983a) it is quite plausible that the dominant individuals get a disproportionately greater share of the food, while expending less energy on foraging. They may thus be able to grow heavier and develop their ovaries, while the less dominant individuals, the workers, suffer from 'nutritional castration'.

The number of females with developed ovaries does not bear any clear relation to the total number of females on the colony; while it shows evidence of an increase with the number of cells in the comb (table 4). Thirty out of 32 nests with less than 100 cells had a single egg-layer, while 14 out of 17 larger nests had 2 or more. The number of cells in a comb is a good indicator of the age of the colony, while the number of females in a colony keeps constantly fluctuating because of periodic large scale emergence and emigrations. We may therefore conclude that the number of egg-layers in a colony increases with the age of the colony. Initially, at the founding, a single female dominates and monopolises

Table 4. Nests with different numbers of egg layers

Number of cells in nest	Frequency of nests with different numbers of females with well developed ovaries (egg-layers)					
	1 egg-layer	2 egg-layers	3 egg-layers	4 egg-layers	5 egg-layers	6 egg-layers
1-100	30	2	1	0	0	0
101-200	2	1	1	1	1	2
201-300	1	0	0	1	0	0
301-400	2	1	0	1	0	0
401-500	0	1	1	0	0	1

all egg-laying ; as the colony develops, this monopoly is broken and other females again the heavier, more dominant ones, also begin to lay eggs.

3.6. Population fluctuations

Nests of *R. marginata* that have long life spans are characterised by continuous fluctuations in the number of adults. Figures 3 and 4 represent the population changes at two nests which grew to a considerable size and lasted for two years or more. In both cases the number of adults on the nests increased initially and following one or more mass emigrations, remained fluctuating for several months at less than 20 adults. In the case of the first nest (figure 3) there were four clear cut instances of mass emigrations. These involved 30 or more adults leaving the nest perhaps to initiate other nests nearby. In the case of the second nest (figure 4), there was a single major exodus, apparently in direct response to predation on the nest by *Vespa tropica*. This large wasp feeds on eggs, larvae and pupae of *R. marginata*. The particular nest depicted in figure 4 was under continual observation, and it is known that the mass exodus followed the first ever visit of the predator to the nest. The predator continued to regularly visit this nest thereafter, and apparently kept the population in check for a year or so. Beyond this period, the nest failed to grow further, although the visits of the predator apparently ceased.

We have rather complete information on population fluctuations at one site in Bangalore where we observed all the nests present at that site for a period of 104 weeks. In all, 35 nests were observed at this study site. The number of nests and the total population of adults and pupae present in all the nests at different times at this site are shown in figure 5. The total population of adults varied between 70 and 400, the population of pupae between 0 and 340 and the total number of nests present at any given time varied between 8 and 16. The largest number of adults were present during January to April in both years. However, the number of pupae and that of the nests seemed to fluctuate rather widely.

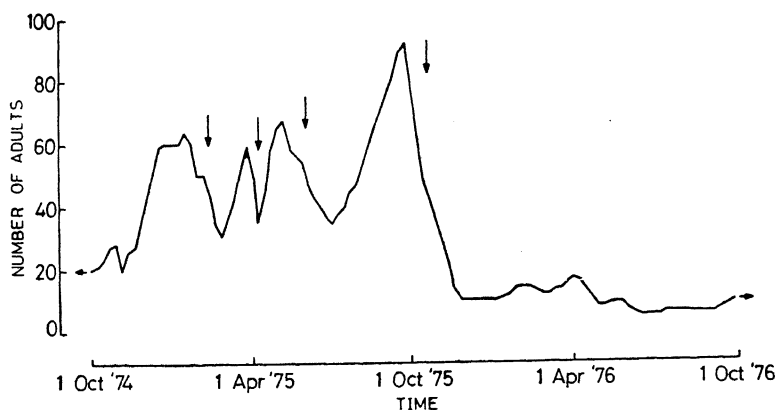


Figure 3. Number of adults at a *Ropalidia marginata* nest in Bangalore. The arrows indicate mass exoduses.

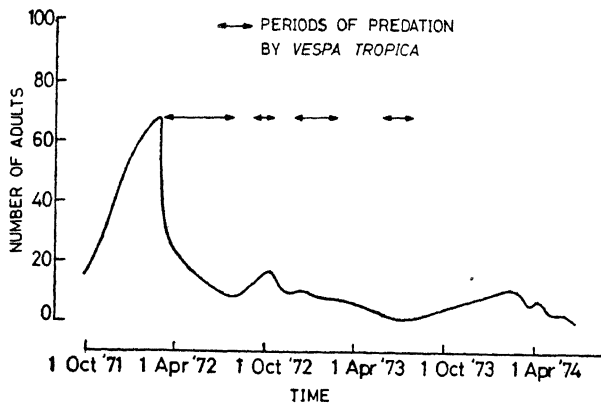


Figure 4. Number of adults at an *R. marginata* nest in Pune. There was a single exodus following the first instance of predation on the nest by *Vespa tropica*. Arrows indicate periods of regular predation by this wasp.

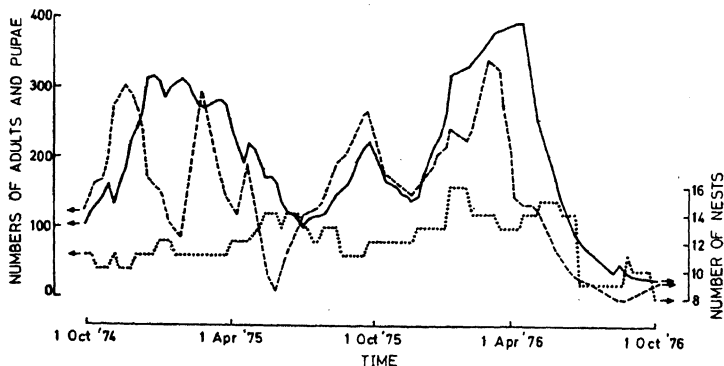


Figure 5. The total number of adults (left ordinate and solid line) and pupae (left ordinate, broken line) and the total number of nests (right ordinate and dotted line) present at different times at a single study site in Bangalore over the period of 104 weeks.

The long lived nests represented in figures 3 and 4 are only a small proportion of the total nests. Most of the nests in fact have a shorter life span. The total life span of 18 nests is known because both the initiation and abandoning of these nests occurred during the period of study. The frequency distribution of total life span of these nests (figure 6A) shows that most nests (70%) have a live span of 10 weeks or less. There was only one nest among these that survived for longer than 30 weeks. However, estimates of the total life spans obtained from any finite period of study is likely to be biased in favour of short lived nests. The distribution of minimum life spans, i.e., where either initiation or abandoning alone were observed (figure 6B) reveals that 10 out of 16 additional nests survived for longer than 30 weeks. Moreover, for one nest neither initiation nor abandoning was observed; in other words, it survived for longer than 104 weeks (the dura-

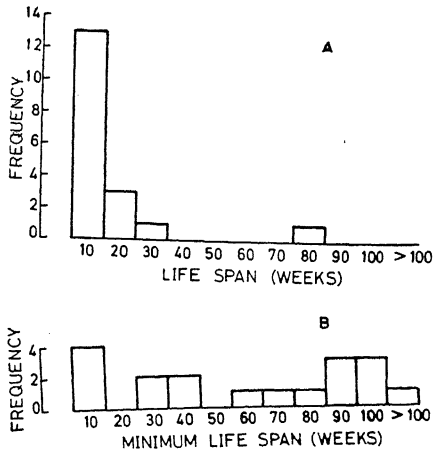


Figure 6. Frequency distribution of total life spans (A) and minimum life spans (B) of *R. marginata* nests. Total life span is defined as the time interval between initiation and abandoning of a nest and is therefore known only for those nests for which both initiation and abandoning occurred during the period of study. Minimum life span is given only for those nests for which either the initiation or abandoning alone is known.

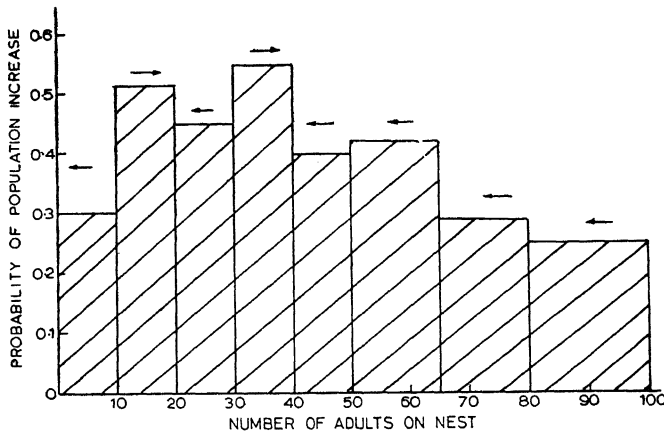


Figure 7. Probability of increase in adult numbers as a function of number of adults already present. The arrows indicate the expected change on the mean in the number of adults in colonies of various sizes.

tion of observation). Thus, although most nests are short lived, some do survive for very long periods of time.

Figure 7 presents further analysis of the population fluctuations. Here we present the probability of increase in the number of adults at a nest as a function of the number of adults already present. These probabilities have been computed by pooling together our data for the 8 nests monitored for over 2 years. As can

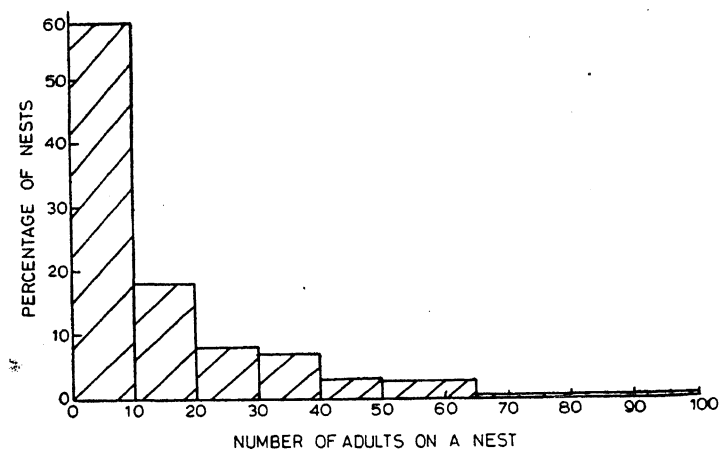


Figure 8. Frequency distribution of the total population of *R. marginata* nests in terms of the number of adults present on the nest.

be seen, the smallest nests have the lowest probability (only 0.3) of further increase in number. They are thus nests most susceptible to extinction. The only sizes at which the nests have near even or better than even chance of increase are between 10 to 40. Thus, a nest which has increased to this level may further increase rapidly till it crosses 40 adults. Beyond this, the nests tend to have a high probability of decrease (due to mass exoduses). The resultant size frequency distribution of nests is presented in figure 8. The vast majority of the nests have less than 10 adults, most go extinct without getting beyond this stage.

4. Discussion

According to the theory of kin selection (Hamilton 1964a, b ; 1972 ; West-Eberhard 1975) the rationale for the development of sociality in ants, bees and wasps lies in their haplodiploid system of sex determination. Because of this, a female wasp is genetically more closely related to her sister than she is to her daughter, and it is therefore more 'advantageous' for a female wasp to help her mother raise daughters which would be her sisters, than to attempt to raise daughters by herself. It is believed that this is why females are selectively favoured to stay on with their mother and help her with the colony labour. At the same time, sons are more closely related to females than brothers are ; hence the workers would have a tendency to lay male eggs, and the males themselves would not share in the colony labour (Hamilton 1964a, b ; Wilson 1971 ; West-Eberhard 1975 ; Trivers and Hare 1976).

Wasp nests with multiple foundresses and multiple egg layers do not fall neatly in this scheme, particularly if the egg-laying females themselves were not close relatives. We however know that in the case of *Polistes* the foundresses do in fact tend to be sisters (West-Eberhard 1969 ; Ross and Gamboa 1981). This system of multiple foundresses can evolve if the nests are highly susceptible to failure in the early stages of growth. Then, if the coming together of several

females increases the probability of success of a nest by a factor of 1.5 or more, sisters may band together, and relinquish reproduction to the most dominant female as the female brood they are raising will be related to them as nieces with coefficient of relatedness = 0.375. If a single female remains reproductive, the workers of the later brood will be raising their sisters with coefficient of relatedness = 0.75.

If, however, more than one of the founding sisters starts to lay, the workers will now be raising a brood related at least in part to them as first cousins coefficient of relatedness = 0.19. At this point the workers may find it more advantageous to leave the nest and attempt to initiate one on their own. This tendency will increase with an increase in the number of egg-layers in the nest.

As discussed earlier, the small nests of *R. marginata* are in fact highly susceptible to failure, hence the banding together of several foundresses is expected. We have no evidence that these are sisters, though this is plausible as new nests are very often founded close to old ones and the foundresses are likely to be sisters who leave together in an exodus from a nest.

We have also shown that there is a tendency for mass exoduses from nests with over 40 adults. This may be related to these being older nests with multiple egg-layers in which the average degree of relationship between the workers and the brood would tend to be low, making it less advantageous for the workers to stay on at nest. Difficulties of sustaining a larger number of adults on the food resources of the home range could be ruled out as a major factor since the new nests are often founded next to the parental nest and must therefore utilize much the same food resources.

In conclusion it appears that ecological pressures render small nests highly susceptible to failure and therefore necessitate the banding together of several females. As the nest grows in size, a single female can no longer dominate it to the level of exclusively monopolizing all egg-laying. With the emergence of multiple egg-layers the workers are at less of an advantage in remaining on the nest and hence begin to leave in significant numbers producing large population fluctuations. An interaction of ecological and social pressures thus determine the course of growth of a nest.

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References

- Alexander R D 1974 The evolution of social behaviour; *Ann. Rev. Ecol. Syst.* 5 325-383
- Belvadi V V and Govindan R 1981 Nesting habits and behaviour of *Ropalidia (Icariola) marginata* (Hymenoptera: Vespidae) in South India; *Colemania* 1 95-101
- Carl J 1934 *Ropalidia montana* sp. et son nid. Un type nouveau d'architecture vespennne; *Rev. Suisse. Zool.* 41 677-691
- Darchen R 1976 *Ropalidia cincta*, guele sociale de la savane de Lamto (Cote-D'Ivoire); *Ann. Soc. Entomol. Fr. (NS)* 12 579-601
- Evans H E and West-Eberhard M J 1970 *The wasps* Ann Arbor, The Univ. of Michigan press: pp. 265

- Gadagkar R 1980 Dominance hierarchy and division of labour in the social wasp, *Ropalidia marginata* (Lep.) (Hymenoptera : Vespidae); *Curr. Sci.* **49** 772-775
- Gadagkar R and Joshi N V 1982a Behaviour of the Indian Social Wasp *Ropalidia cyathiformis* (Fab.) on a nest of separate combs (Hymenoptera : Vespidae); *J. Zool.* **198** 27-37
- Gadagkar R and Joshi N V 1982b A Comparative study of social structure in colonies of *Ropalidia*, in *The Biology of the Social Insects*, (eds) M D Breed, C D Michener and H E Evans, Proc. IX congress of the International Union for the study of social Insects, Boulder, Colorado USA pp. 187-191
- Gadagkar R and Joshi N V 1982c When a wasp colony splits In *The Biology of the Social Insects*, (eds) M D Breed, C D Michener and H E Evans Proc. IX congress of the International union for the Study of Social Insects, Boulder Colorado USA pp. 217
- Gadagkar R and Joshi N V 1983a Quantitative ethology of social wasps : Time-activity budgets and caste differentiation in *Ropalidia marginata* (Lep.) (Hymenoptera : Vespidae); *Animal Behaviour* **31** 26-31
- Gadagkar R and Joshi N V 1983b Social organization in the Indian wasp *Ropalidia cyathiformis* (Fab.) (Hymenoptera : Vespidae); *Z. Tierpsychol.* (in Press)
- Gadgil M and Mahabal A S 1974 Caste differentiation in the paper wasp *Ropalidia marginata* (Lep.); *Curr. Sci.* **43** 482
- Hamilton W D 1964a The genetical evolution of social behaviour I; *J. Theor. Biol.* **7** 1-16
- Hamilton W D 1964b The genetical evolution of social behaviour II; *J. Theor. Biol.* **7** 17-52
- Hamilton W D 1972 Altruism and related phenomena mainly in the social insects; *Ann. Rev. Ecol. Syst.* **3** 193-232
- Jeanne R L 1972 Social biology of the Neo-tropical wasp *Mischocyttarus drewseni*; *Bull. Mus. Comp. Zool. Harv. Univ.* **144** 63-150
- Jeanne R L 1980 Evolution of social behaviour in the Vespidae; *Ann. Rev. Entomol.* **25** 371-396
- Lin N and Michener C D 1972 Evolution of sociality in insects; *Q. Rev. Biol.* **47** 131-159
- Little M 1977 Behavioural ecology of the social wasp, *Mischocyttarus mexicanus*; *Beh. Ecol. Sociobiol.* **2** 229-246
- Little M 1979 *Mischocyttarus flavitarsis* in Arizona : social and nesting biology of a polistine wasp; *Z. Tierpsychol.* **50** 282-312
- Little M 1981 Social biology of the Polistine wasp *Mischocyttarus labiatus* : survival in a Colombian rain forest; *Smiths. Contr. Zool. No.* 327
- Michener C D 1969 Comparative social behaviour of bees; *Ann. Rev. Entomol.* **14** 299-342
- Michener C D 1974 *The social behaviour of the bees*; Massachusetts : Harvard University Press xii + 404 pp.
- Richards O W and Richards M J 1951 Observations on the social wasps of South America (Hymenoptera : Vespidae); *Trans. Roy. Entomol. Soc. Lond.* **102** 1-170
- Ross N M and Gamboa G J 1981 Nestmate discrimination in social wasps (*Polistiers metricus*, Hymenoptera : Vespidae); *Beh. Ecol. Sociobiol.* **9** 163-165
- Roubaud E 1916 Recherches biologiques sur les guêpes sociales et cosales d'Afrique. La guêpe de la vie sociale et l'évolution de l'instinct maternel chez les vespides; *Ann. Sci. Nat. Zool.* **1** 1-160
- Saussure H de 1853-59 *Monographie de guêpes sociales, ou de la tribu des Vespiciens, ouvrage faisant suite à la monographie des guêpes solitaires* V. Masson Paris
- Starr C K 1979 Origin and evolution of insect sociality; A review of modern theory; In *Social Insects*, (ed) H R Hermann (New York : Academic Press) Vol. I pp. 35-79
- Trivers R L and Hare H 1976 Haplodiploidy and the evolution of the social insects; *Science* **191** 249-263
- Vecht J Van der 1962 The Indo-Australian species of the genus *Ropalidia* (*Icaria*) (Second part); *Zool. Verhand. Leiden* **57** 1-72
- West-Eberhard M J 1969 Social biology of polistine wasps; *Misc. Publ. Mus. Zool. Univ. Mich.* **140** 1-101
- West-Eberhard M J 1975 The evolution of social behaviour by kin selection; *Q. Rev. Biol.* **50** 1-33
- Wilson E O 1971 *The insect societies*; Massachusetts : Harvard University Press x + 548 pp.
- Wilson E O 1975 *Sociobiology*; Massachusetts : Harvard University Press ix + 697 pp.

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Abstract. The Spongillid *Corvospongilla lapidosa* (Annandale 1908) (Porifera : Spongillidae) is reported for the first time from the Manjira reservoir in Andhra Pradesh. Its sausage shaped spicules have adaptive value to thrive in low silica environments. The species is tolerant to high turbidity. High calcium and bicarbonate may be unfavourable and the sponge has not been found either on molluscan shells or on aquatic vegetation. It can thrive in waters more than 4m deep. The range and mean values of twenty-seven physico-chemical parameters of the habitat of *C. lapidosa* are given as the base data for the species ecology.

Keywords. Ecology ; Porifera ; *Corvospongilla lapidosa* ; Manjira reservoir ; silica ; turbidity ; spicules.

1. Introduction

In the aquatic ecosystems poriferan fauna occupying the benthic habitat constantly circulate water in their elaborate and complex canal system through their multiporous body surface. So it is reasonable to expect that in the lentic and lotic bodies of fresh-water the spongillid species play an important but yet unrecognized role in the cycling of abiotic and biotic substances. Apart from the pioneering work and important contributions on the taxonomy and geographical distribution by Annandale (1907, 1908, 1909, 1909a, 1911, 1912, 1913 and 1915) and subsequent supplemental work of Gist (1930, 1932) the Indian spongillids had to remain neglected until Tonapi (1964) added to their list of habitats of earlier known species. There is no record of the environmental parameters of the fresh-water bodies in which the Indian spongillids colonized except for the ecologically poor descriptions of their habitats as clear, dirty, turbid, polluted waters etc. In the course of a two year study from February 1979 through January 1981, on the ecology of the Manjira reservoir formed by a man-made barrage (17° 39' N, 78° 04' E) located near Sangareddy, Andhra Pradesh, when spicules were frequently encountered in the samples of plankton, search was undertaken to locate the habitats of the sponges. During these studies a number of specimens of

the spongillid species, *Corvospongilla lapidosa* (Annandale 1908) (family : Spongillidae) were discovered and observations made on certain aspects of its ecobiology are discussed in this paper.

2. The river Manjira and its reservoir

The river Manjira, a tributary of the Godavari in South India has a barrage constructed across it near Sangareddy (17° 37' N, 78° 06' E) to form a potable water reservoir (figure 1) to supply the city of Hyderabad. The reservoir has a catchment area of about 16,780 km² and a maximum storage capacity of 73·63 Mm³. Due to heavy siltation the maximum depth of the reservoir near the barrage is now about 9·5 m. The river bed is dotted with boulders of various sizes some of them buried deep in the silty clay while a few lie exposed during the depletion of water level in summer.

3. Materials and methods

Surfacial water samples (2·5 l) from four stations (I, II, III and IV—figure 1) bathymetric water samples (2·4 l) from 3 m, 6 m and 0·5 m near the bottom of the reservoir (V, VI and VII stations—figure 1) operating a closing type of bottle sampler (1·2 l capacity) twice near station III were collected monthly for two years from February 1979. Temperature of water was read in the field by using a mercury thermometer (0°–110° C). Secchi disc (Welch 1948) was used near station III for determining the Zsd. In a field laboratory near the barrage pH and conductivity were determined by using conductivity–pH meter (CLO1/03 Toshniwal). Carbonic species of water were estimated by titrimetry and the samples were then brought to the Department of Zoology, Osmania University, Hyderabad for further analyses of various chemical parameters according to APHA AWWA and WPCF (1971).

4. Observations

4.1 Habitat

During the summer months of May and June of 1979 and 1980 the water level in the reservoir receded considerably exposing a number of boulders, cobbles and pebbles in the reservoir bed in the vicinity of the sampling stations I and IV. These provided the substratum for the encrustation of the sponge, *Corvospongilla lapidosa*. In summer the exposed reservoir bed presented dry caked mud that is extensively fissured to a depth of 0·4 to 0·5 m. During winter and rainy seasons, the littoral region of the reservoir supports fairly rich emergent growth of aquatic plants *Typha angustata* and *Scirpus littoralis* and submerged species of *Potamogeton perfoliatus* and *Vallisneria spiralis*. The depth of the littoral varies from 0·5 to 1·5 m. There are no trees on the margins of the reservoir to impart any shade.

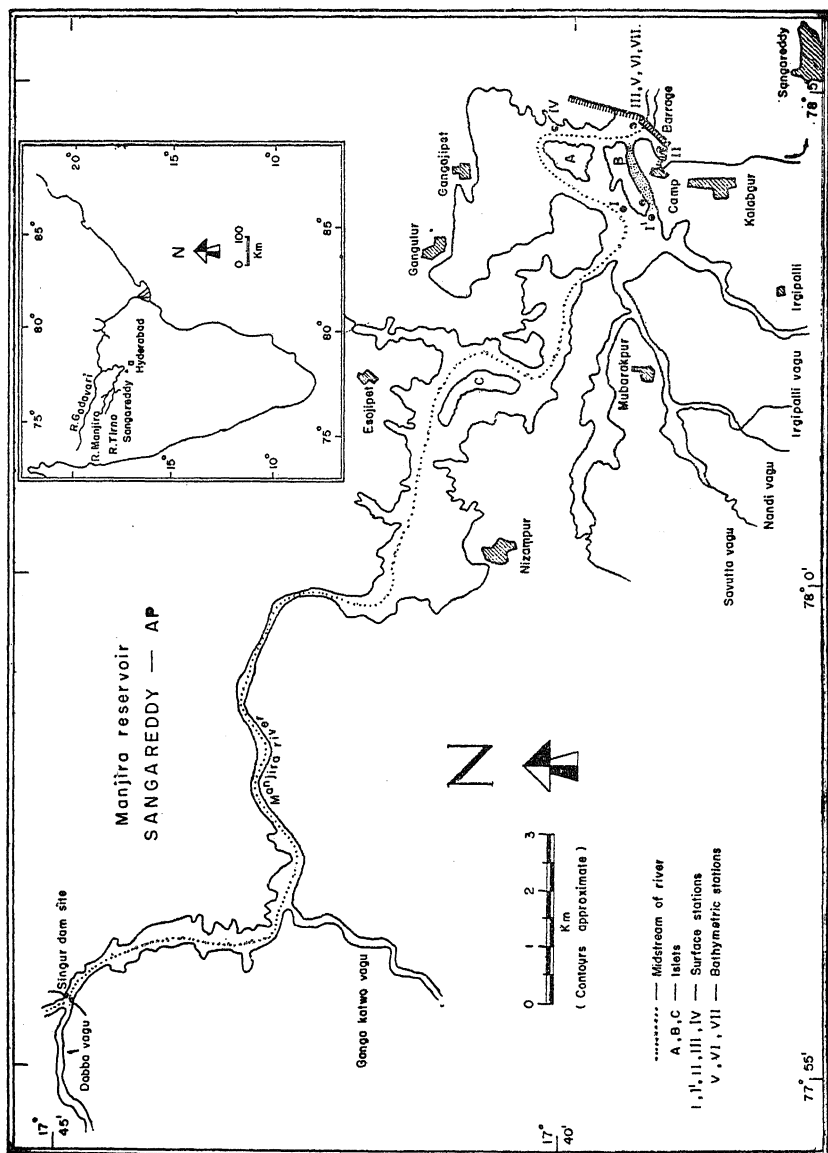


Figure 1. Habitat location of *Corvospongilla* in the Manjira reservoir, showing sampling stations I-VII.

4.2. Ecomorphic characters

Corvospongilla grows on large boulders covering their exposed surfaces as a more or less flat sheet of 0.6 to 2.0 cm thickness (figure 2a). On small cobbles and pebbles (figure 3a) the thickness of the sponge is reduced to 0.3 to 1.0 cm. The sponge body is steel grey or bluish black in colour. Near Station III the barrage wall of the north flank was also found encrusted with this sponge which is yellowish brown in colour. The water at this station is 6 to 9.5 m but usually 8 m deep and devoid of angiospermic vegetation.

Microscopic examination of the sponge body (figure 2) reveals the appearance of a corrugated body surface with sausage shaped amphistrongylous spicules lying embedded on the surface in different directions. Oscula are inconspicuous, dispersed at random, some raised on irregular eminences. The thick chitinous membrane at the base of the sponge body is tough and shows a few furrows probably made by worms. The structure, shape and size of the spicules (figure 3b, c) conform to the descriptions of Annandale (1911) for the species.

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According to Khera and Chaturvedi (1976) the distribution of *Corvospongilla lapidosa* is Maharashtra—Igatpuri lake, the river Godavari at Nasik ; Karnataka—Bangalore ; Bihar—Santhal Paragana ; West Bengal—Barrackpore. Tonapi (1964) reported it from small rivers near Poona. The occurrence of this species of spongillid in the Manjira reservoir is a new record for this river and also for Andhra Pradesh. Schizotype of this species was sent to Zoological Survey of India, Calcutta and the identification was confirmed.

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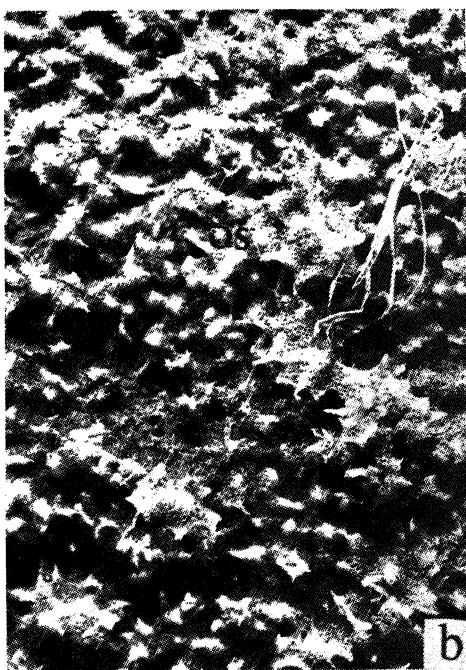
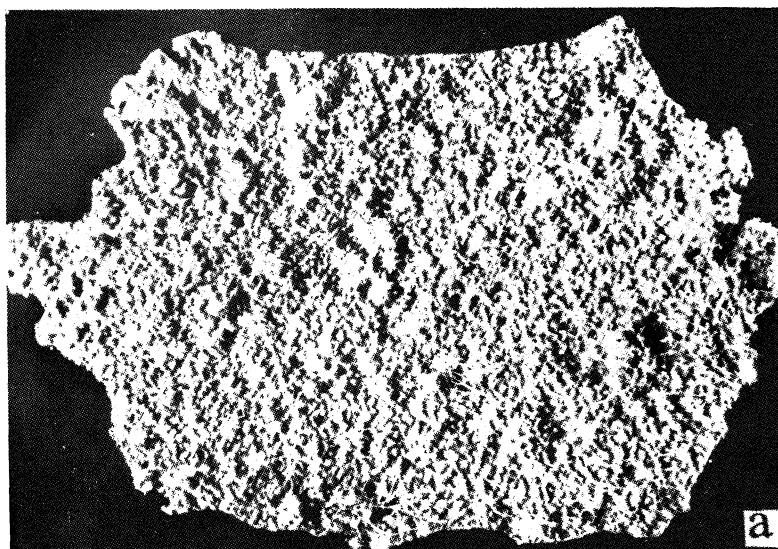


Figure 2. a. Dried sheet of *Corvospongilla* from a boulder (surface view). b. Magnified surface view showing Oscula (os). c. Undersurface of the sponge showing chitinous membrane and worm furrows.

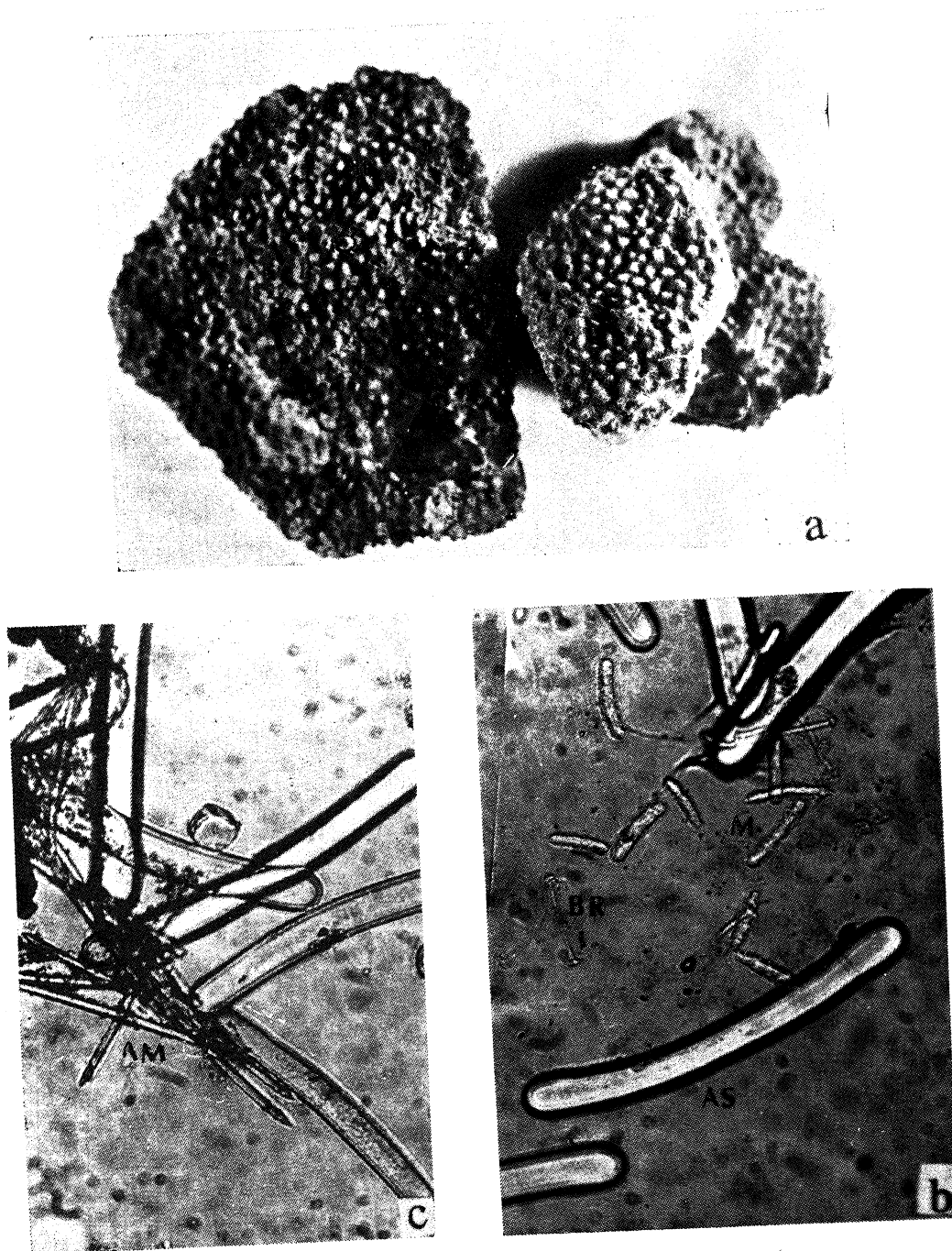


Figure 3. a. *Corvospongilla* on a cobble (left) and pebble (right). b and c. spicules—amphistrongyli (AS); microscleres (M): birotulates (BR); amphioxi (AM).

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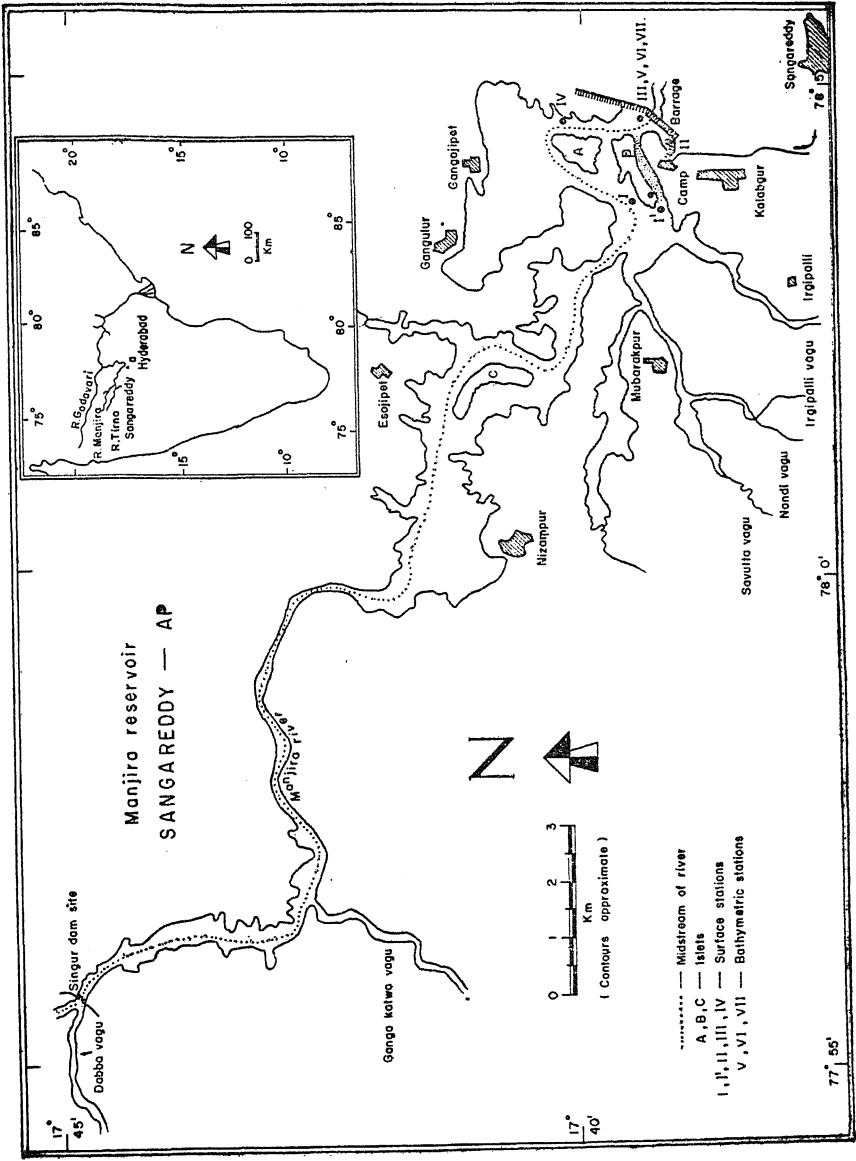


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Table 1. Stationwise and yearwise mean values of certain physico-chemical parameters—Manjira reservoir.

Parameters	Stations						
	I	II	III	IV	V	VI	VII
Calcium (mg/l)	17·302	22·675	16·498	17·402	15·164	15·115	14·563
	20·842	22·735	17·568	17·269	15·431	13·780	14·569
Bicarbonate (mg/l)	203·07	217·36	201·75	190·18	198·01	202·10	209·71
	180·52	193·71	178·69	161·80	174·77	179·60	177·33
Silica (mg/l)	2·042	0·983	1·854	1·813	1·833	1·833	2·229
	1·813	1·718	1·917	1·792	1·750	1·771	1·850
Turbidity	41	6·75	47	50	82	101	111
(Hydrazine units)	71	35	43	79	58	84	70

(Values in the upper row are for 1979-80 while those of lower row are for 1980-81)

by the quantity of available silica in waters. Jewell (1935) reported that *Spongilla lacustris* living in water of silica content below 0·4 mg/l and low conductivity and solids shows progressive attenuation of its spicules, while marked variations occurred in another species, *Tubella pennsylvanica*. But *Corvospongilla* shows the preponderance of thin, hollow, sausage-shaped spicules over the relatively few small isolated groups of birotulate and amphioxi spicules in its body. Apparently these spicules have adaptive value to exploit the low silica environment of the Manjira reservoir and interestingly it is the only species occurring in this reservoir. The sausage shaped spicules offered greater surface area per unit mass of available body silica and thereby enhanced the surface area of the body to increase the ability to absorb the scarce silica from the ambient environment. The silica-lemma of the developing spicule is known to be the site of inward transport of silica for polymerizing it on its inner surface (Harrison and Cowden 1976). The competition of *C. lapidosa* for the leachable silica is one of the factors for the poor standing crop of planktonic diatoms in this reservoir (Rao 1982).

The water of the Manjira reservoir is prone to sudden, short but fairly frequent spells of moderately high turbidity even though the mean values in table 1 suggest that the water is optically less turbid. Excluding the II station, 33% of our data points, showed more than 90 Hydrazine units of turbidity. Import of silt from the catchment during floods, wind generated turbulence, water discharge across the barrage cause high turbidity in this shallow reservoir. Abnormal values of 660, 3080, 2640 and 3800 units of turbidity were recorded at stations III, V, VI and VII respectively on 14 June 1980 when four out of eleven flood gates were raised to flush a part of the silt in the reservoir and even then this species was found to survive.

As this species is found covering the entire exposed surface of the boulders and even small stones, backwashing (Storr 1976) and amoebocyte scavenging activity (Harrison 1974) must be at work in this spongillid to prevent the silt from

covering the body surface and clog the canal system. The low profile of the body and the loosely architected form in this species and the apparent flexibility of the spicules are no doubt helpful in such an endeavour of the animal in tolerating a wide range of turbidity.

The species ecology of North American spongillids (Harrison 1974, 1977 ; Harrison *et al* 1977 ; Harrison and Harrison 1979) provided the basis for possible use of sponges as indicators of pollution. The paucity of data on the ecological parameters of the Indian spongillids precludes, for the present, comparative evaluation of the species ecology of *C. lapidosa* with its other habitats and with other spongillids of the Indian subcontinent. Meanwhile in the absence of any point source of anthropogenic pollution at the Manjira reservoir, the physico-chemical conditions of the habitat of *C. lapidosa* (table 2) provide the base data.

Table 2. Physico-chemical parameters of the Manjira reservoir habitat of *Corvospongilla lapidosa*

Parameter	Data points	Min.	Max.	Mean
Water temperature (°C)	165	19.10	34.00	27.73
pH	165	7.60	9.35	8.40
Conductivity (wS/cm at 25° C)	102	90	390	193.55
Secchi disc depth (Z_{sdm})	48	0.030	1.065	0.472
Turbidity (Hydrazine units)	161	1.6	386*	63
Suspended solids (mg/l)	161	0.0	456.0*	71.388
Dissolved solids (mg/l)	165	96.0	417.0	236.756
Oxidizable organic matter as O ₂ absorbed from KMnO ₄ (incubated for 3 hours at 37° C)	165	0.00	12.72	2.801
Dissolved oxygen (mg/l)	165	2.45	18.59	8.774
Carbon dioxide (mg/l)	165	0.00	14.52	1.02
Carbonate alkalinity (mg/l)	165	0.00	57.00	14.375
Bicarbonate alkalinity (mg/l)	165	88.48	314.25	190.528
Chlorides (mg/l)	165	9.96	54.95	22.418
Sulphates (mg/l)	165	0.00	39.25	18.896
Silica (mg/l)	165	0.40	6.00	1.74
Ammonia nitrogen (mg/l)	165	0.000	3.944	0.4915
Albuminoid nitrogen (mg/l)	165	0.000	3.697	0.6775
Nitrite nitrogen (mg/l)	165	0.0000	0.0186	0.0026
Nitrate nitrogen (mg/l)	165	0.000	0.750	0.0945
Total nitrogen (mg/l)	165	0.0022	7.1271	1.2647
Orthophosphate phosphorus (mg/l)	165	0.000	0.90	0.0176
Total phosphorus (mg/l)	165	0.000	0.190	0.0394
Sodium (mg/l)	165	6.5	135.0	50.595
Potassium (mg/l)	165	1.7	4.9	2.85
Calcium (mg/l)	165	3.21	32.06	17.212
Magnesium (mg/l)	165	0.2	54.0	16.232
Total iron (mg/l)	165	0.00	1.32	0.1465

(* Abnormal values when two or more flood gates are temporarily lifted are ignored)

On this evidence, it can be broadly concluded that *C. lapidosa* can inhabit rocky substrata in alkaline, and turbid waters with low dissolved silica.

Acknowledgements

One of us (ISR) is thankful to the University Grants Commission, New Delhi, for award of fellowship under Faculty Improvement Programme and to the Head of the Department of Zoology, Osmania University for laboratory facilities. Thanks are due to Dr G C Rao, zsi, Calcutta, for confirming the identification and to Dr M Babu Rao, zsi, Hyderabad, for useful suggestions and library facilities. Grateful acknowledgements are to the Superintending Engineer, Manjira water works circle for relevant hydrographic data and permission to collect water samples and to Sri G Goutham Reddy, Assistant Engineer, for excellent cooperation and help in the field work. Thanks are to Dr M Mahmood for photography.

References

- Allee W C, Emerson A E, Park O, Park T and Schmidt K P 1955 *Principles of Animal Ecology* (London : W B Saunders Co.) p. 204
- Annandale N 1907 On fresh water sponges from Calcutta and the Himalayas ; *J. Asiatic Soc. Bengal* (n.s.) 3 15-26
- Annandale N 1908 Preliminary notice of a collection of sponges from West India with descriptions of two new species ; *Rec. Indian Mus.* 2 25-28
- Annandale N 1909 Report on a small collection of sponges from Travancore ; *Rec. Indian Mus.* 3 101-104
- Annandale N 1909a Description of a new species of *Spongilla* from Orissa ; *Rec. Indian Mus.* 3 275
- Annandale N 1911 The Fauna of British India including Ceylon and Burma Freshwater sponges, Hydroids and Polyzoa (London : Taylor and Francis) 1-251
- Annandale N 1912 Fresh-water sponges of Malabar zone ; *Rec. Indian Mus.* 7 383-398
- Annandale N 1913 Notes on freshwater sponges No. XI Sponges from the shells of Aetheria ; *Rec. Indian Mus.* 9 237-240
- Annandale N 1915 Notes on freshwater sponges No. XVI. The genus *Pectispongilla* and its allies ; *Rec. Indian Mus.* 11 171-178
- APHA, AWWA and WPCF 1971 Standard methods for the examination of water and waste water, 13 edition, (eds.) M J Taras, A E Greenberg, R D Hoak and M C Rand American Public Health Association, Washington
- Gist G N 1930 Notes on the Freshwater sponge *Trochospongilla phillottiana* and its varieties ; *Rec. Indian Mus.* 32 491-495
- Gist G N 1932 *Spongilla carteri* and its varieties ; *Rec. Indian Mus.* 34 185-195
- Harrison F W 1974 Sponges (Porifera : Spongillidae). In Pollution ecology of freshwater invertebrates, (eds) C W Hart, S L H Fuller (New York and London : Academic Press) 29-66
- Harrison F W 1977 The taxonomic and ecological status of the environmentally restricted spongillid species of North America. III. *Corvomeyenia carolinensis* Harrison 1971 ; *Hydrobiologia* 56 187-190
- Harrison F W and Cowden R R 1976 (eds) *Aspects of sponge biology* (New York and London : Academic Press) 40-43
- Harrison F W and Harrison M B 1979 Taxonomic and ecological status of environmentally restricted spongillid species of North America. IV. *Spongilla heterosclerifera* Smith 1918 ; *Hydrobiologia* 62 107-111

- Harrison F W, Johnston L, Stansell K B and McAndrew W 1977 The taxonomic and ecological status of the environmentally restricted spongillid species of North America. 1. *Spongilla sponginosa* Penny 1957 ; *Hydrobiologia* **53** 199-202
- Jewell M E 1935 An ecological study of the fresh-water sponges of Northern Wisconsin ; *Eco. Monogr.* **5** 461-504
- Jewell M E 1939 An ecological study of the freshwater sponges of Wisconsin. II. The influence of Calcium ; *Ecology* **20** 11-28
- Khera S and Chaturvedi Y 1976 Check-list of Indian freshwater sponges ; *Rec. Zool. Surv. India Occ. paper* No. 4 pp. 29
- Rao I S 1982 *Ecology of the Manjira reservoir* ; Ph.D. thesis (submitted)
- Storr J F 1976 Field observations of sponge reactions as related to their ecology. In *Aspects of Sponge biology* (eds.) F W Harrison and R R Cowden (New York : Academic Press) 277-282
- Tonapi G T 1964 A note on the freshwater sponges of Poona ; *Curr. Sci.* **33** 372-373
- Welch P S 1948 *Limnological methods* (London : McGraw-Hill Book Co. Inc.) 159-160

Table 1. Stationwise and yearwise mean values of certain physico-chemical parameters—Manjira reservoir.

Parameters	Stations						
	I	II	III	IV	V	VI	VII
Calcium (mg/l)	17·302	22·675	16·498	17·402	15·164	15·115	14·563
	20·842	22·735	17·568	17·269	15·431	13·780	14·569
Bicarbonate (mg/l)	203·07	217·36	201·75	190·18	198·01	202·10	209·71
	180·52	193·71	178·69	161·80	174·77	179·60	177·33
Silica (mg/l)	2·042	0·983	1·854	1·813	1·833	1·833	2·229
	1·813	1·718	1·917	1·792	1·750	1·771	1·850
Turbidity	41	6·75	47	50	82	101	111
(Hydrazine units)	71	35	43	79	58	84	70

(Values in the upper row are for 1979-80 while those of lower row are for 1980-81)

by the quantity of available silica in waters. Jewell (1935) reported that *Spongilla lacustris* living in water of silica content below 0·4 mg/l and low conductivity and solids shows progressive attenuation of its spicules, while marked variations occurred in another species, *Tubella pennsylvanica*. But *Corvospongilla* shows the preponderance of thin, hollow, sausage-shaped spicules over the relatively few small isolated groups of birotulate and amphioxi spicules in its body. Apparently these spicules have adaptive value to exploit the low silica environment of the Manjira reservoir and interestingly it is the only species occurring in this reservoir. The sausage shaped spicules offered greater surface area per unit mass of available body silica and thereby enhanced the surface area of the body to increase the ability to absorb the scarce silica from the ambient environment. The silica-lemma of the developing spicule is known to be the site of inward transport of silica for polymerizing it on its inner surface (Harrison and Cowden 1976). The competition of *C. lapidosa* for the leachable silica is one of the factors for the poor standing crop of planktonic diatoms in this reservoir (Rao 1982).

The water of the Manjira reservoir is prone to sudden, short but fairly frequent spells of moderately high turbidity even though the mean values in table 1 suggest that the water is optically less turbid. Excluding the II station, 33% of our data points, showed more than 90 Hydrazine units of turbidity. Import of silt from the catchment during floods, wind generated turbulence, water discharge across the barrage cause high turbidity in this shallow reservoir. Abnormal values of 660, 3080, 2640 and 3800 units of turbidity were recorded at stations III, V, VI and VII respectively on 14 June 1980 when four out of eleven flood gates were raised to flush a part of the silt in the reservoir and even then this species was found to survive.

As this species is found covering the entire exposed surface of the boulders and even small stones, backwashing (Storr 1976) and amoebocyte scavenging activity (Harrison 1974) must be at work in this spongillid to prevent the silt from

covering the body surface and clog the canal system. The low profile of the body and the loosely architected form in this species and the apparent flexibility of the spicules are no doubt helpful in such an endeavour of the animal in tolerating a wide range of turbidity.

The species ecology of North American spongillids (Harrison 1974, 1977 ; Harrison *et al* 1977 ; Harrison and Harrison 1979) provided the basis for possible use of sponges as indicators of pollution. The paucity of data on the ecological parameters of the Indian spongillids precludes, for the present, comparative evaluation of the species ecology of *C. lapidosa* with its other habitats and with other spongillids of the Indian subcontinent. Meanwhile in the absence of any point source of anthropogenic pollution at the Manjira reservoir, the physico-chemical conditions of the habitat of *C. lapidosa* (table 2) provide the base data.

Table 2. Physico-chemical parameters of the Manjira reservoir habitat of *Corvospongilla lapidosa*

Parameter	Data points	Min.	Max.	Mean
Water temperature (°C)	165	19.10	34.00	27.73
pH	165	7.60	9.35	8.40
Conductivity (wS/cm at 25° C)	102	90	390	193.55
Secchi disc depth (Z_{sam})	48	0.030	1.065	0.472
Turbidity (Hydrazine units)	161	1.6	386*	63
Suspended solids (mg/l)	161	0.0	456.0*	71.388
Dissolved solids (mg/l)	165	96.0	417.0	236.756
Oxidizable organic matter as O ₂ absorbed from KMnO ₄ (incubated for 3 hours at 37° C)	165	0.00	12.72	2.801
Dissolved oxygen (mg/l)	165	2.45	18.59	8.774
Carbon dioxide (mg/l)	165	0.00	14.52	1.02
Carbonate alkalinity (mg/l)	165	0.00	57.00	14.375
Bicarbonate alkalinity (mg/l)	165	88.48	314.25	190.528
Chlorides (mg/l)	165	9.96	54.95	22.418
Sulphates (mg/l)	165	0.00	39.25	18.896
Silica (mg/l)	165	0.40	6.00	1.74
Ammonia nitrogen (mg/l)	165	0.000	3.944	0.4915
Albuminoid nitrogen (mg/l)	165	0.000	3.697	0.6775
Nitrite nitrogen (mg/l)	165	0.0000	0.0186	0.0026
Nitrate nitrogen (mg/l)	165	0.000	0.750	0.0945
Total nitrogen (mg/l)	165	0.0022	7.1271	1.2647
Orthophosphate phosphorus (mg/l)	165	0.000	0.90	0.0176
Total phosphorus (mg/l)	165	0.000	0.190	0.0394
Sodium (mg/l)	165	6.5	135.0	50.595
Potassium (mg/l)	165	1.7	4.9	2.85
Calcium (mg/l)	165	3.21	32.06	17.212
Magnesium (mg/l)	165	0.2	54.0	16.232
Total iron (mg/l)	165	0.00	1.32	0.1465

(* - Abnormal values when two or more flood gates are temporarily lifted are ignored)

On this evidence, it can be broadly concluded that *C. lapidosa* can inhabit rocky substrata in alkaline, and turbid waters with low dissolved silica.

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References

- Allee W C, Emerson A E, Park O, Park T and Schmidt K P 1955 *Principles of Animal Ecology* (London : W B Saunders Co.) p. 204
- Annandale N 1907 On fresh water sponges from Calcutta and the Himalayas ; *J. Asiatic Soc. Bengal* (n.s.) 3 15-26
- Annandale N 1908 Preliminary notice of a collection of sponges from West India with descriptions of two new species ; *Rec. Indian Mus.* 2 25-28
- Annandale N 1909 Report on a small collection of sponges from Travancore ; *Rec. Indian Mus.* 3 101-104
- Annandale N 1909a Description of a new species of *Spongilla* from Orissa ; *Rec. Indian Mus.* 3 275
- Annandale N 1911 The Fauna of British India including Ceylon and Burma Freshwater sponges, Hydroids and Polyzoa (London : Taylor and Francis) 1-251
- Annandale N 1912 Fresh-water sponges of Malabar zone ; *Rec. Indian Mus.* 7 383-398
- Annandale N 1913 Notes on freshwater sponges No. XI Sponges from the shells of Aetheria ; *Rec. Indian Mus.* 9 237-240
- Annandale N 1915 Notes on freshwater sponges No. XVI. The genus *Pectispongilla* and its allies ; *Rec. Indian Mus.* 11 171-178
- APHA, AWWA and WPCF 1971 Standard methods for the examination of water and waste water, 13 edition, (eds.) M J Taras, A E Greenberg, R D Hoak and M C Rand American Public Health Association, Washington
- Gist G N 1930 Notes on the Freshwater sponge *Trochospongilla phillottiana* and its varieties ; *Rec. Indian Mus.* 32 491-495
- Gist G N 1932 *Spongilla carteri* and its varieties ; *Rec. Indian Mus.* 34 185-195
- Harrison F W 1974 Sponges (Porifera : Spongillidae). In Pollution ecology of freshwater invertebrates, (eds) C W Hart, S L H Fuller (New York and London : Academic Press) 29-66
- Harrison F W 1977 The taxonomic and ecological status of the environmentally restricted spongillid species of North America. III. *Corvomeyenia carolinensis* Harrison 1971 ; *Hydrobiologia* 56 187-190
- Harrison F W and Cowden R R 1976 (eds) *Aspects of sponge biology* (New York and London : Academic Press) 40-43
- Harrison F W and Harrison M B 1979 Taxonomic and ecological status of environmentally restricted spongillid species of North America. IV. *Spongilla heterosclerifera* Smith 1918 ; *Hydrobiologia* 62 107-111

- Harrison F W, Johnston L, Stansell K B and McAndrew W 1977 The taxonomic and ecological status of the environmentally restricted spongillid species of North America. 1. *Spongilla sponginosa* Penny 1957 ; *Hydrobiologia* **53** 199-202
- Jewell M E 1935 An ecological study of the fresh-water sponges of Northern Wisconsin ; *Eco. Monogr.* **5** 461-504
- Jewell M E 1939 An ecological study of the freshwater sponges of Wisconsin. II. The influence of Calcium ; *Ecology* **20** 11-28
- Khera S and Chaturvedi Y 1976 Check-list of Indian freshwater sponges ; *Rec. Zool. Surv. India Occ. paper* No. 4 pp. 29
- Rao I S 1982 *Ecology of the Manjira reservoir* ; Ph.D. thesis (submitted)
- Storr J F 1976 Field observations of sponge reactions as related to their ecology. In *Aspects of Sponge biology* (eds.) F W Harrison and R R Cowden (New York : Academic Press) 277-282
- Tonapi G T 1964 A note on the freshwater sponges of Poona ; *Curr. Sci.* **33** 372-373
- Welch P S 1948 *Limnological methods* (London : McGraw-Hill Book Co. Inc.) 159-160

Seasonal fluctuations in the diet composition of *Rhinopoma hardwickei* in the Rajasthan desert

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Abstract. The small mouse-tailed bat, *Rhinopoma hardwickei*, collected from various districts of Rajasthan, is primarily an insectivorous species. Orthoptera, Dictyoptera, Lepidoptera, Hymenoptera, Coleoptera and Diptera are preferred in all main four seasons in varying amounts, while Isoptera are consumed in all but the winter season. Occurrence of ground dwelling insects, caterpillars, spiders and water beetles in the stomachs of bats have been discussed in the light of behavioural adaptations of this species. Presence of fur of same bat species in stomachs coincides with its breeding season. Presence of various polyphagous insect pest species of crops in feeding menu of bats shows that this species plays an important role in biological management of harmful insects.

Keywords. *Rhinopoma* ; diet composition ; biological management ; insect pest.

1. Introduction

The small mouse-tailed bat, *Rhinopoma hardwickei* Gray, 1831 (Chiroptera : Rhinopomatidae) is a fairly well distributed species in Rajasthan which is part of the Great Indian *Thar* desert (24·5-30·5° N ; 60-70° E). Associated with arid and semi arid regions, of which it is adapted ecophysiologically, this species is confined to subtropical latitudes. In the Indian subcontinent this bat is absent from forested regions of Ghats. In its diurnal roost, it coexists with other bat species of *Rhinopoma microphyllum kinneari* and *Taphozous* spp. inhabiting natural caves, man made cellars, and underground irrigation tunnels.

In spite of the occurrence of *R. hardwickei* in abundance, constituting of 9·63 % of the total bat fauna of desert biome of Rajasthan (Advani 1981a), except some reports (Advani and Vazirani 1981 ; Prakash 1963 ; Sinha and Advani 1976), little is known about the ecology, biology and behaviour of this species. The present studies were undertaken to investigate the food composition and seasonal variation in the feeding pattern of this species.

2. Study area

The Indian desert has four distinct seasons in one year, receiving different magnitudes of rainfall, temperature fluctuations, relative humidity and sun shine hours. These factors individually and/or combinely have an impact on reproduction, abundance and activity of animal life in the desert including insects and bats. In winter, the mean maximum and minimum temperatures are 25.4 and 9.5° C respectively, with a mean rainfall of 2.8 mm and a mean relative humidity of 22% (Prakash *et al* 1971). In summer, the temperature is very high (mean max t = 39.8° C, mean min t = 27.9° C), with 18.2 mm mean rainfall and 20.1 mean relative humidity. In the monsoon months, the mean rainfall is 110.5 mm, relative humidity being 47.5% and the temperature (mean max t = 34.5° C, mean min. t = 25.1° C) lesser than that of summer. In post-monsoon season (October and November) the temperature fluctuations are 28.5° C (max.) to 10.8° C (min). The relative humidity is 20.5%.

3. Materials and methods

The bats were collected during various seasonal and periodical faunistic surveys conducted by Desert Regional Station, Zoological Survey of India of twelve districts—Jodhpur, Barmer, Nagaur, Pali, Dungarpur, Banswara, Jhalawar, Tonk, Boondi, Ajmer, Sawai Madhopur and Kota, well distributed in arid and semi arid parts of Rajasthan State. 171 individuals were collected and examined. For each season, the break up of the sample size (N) is shown in table 1. After anaesthesia the bats were dissected and their alimentary canals cut open. The stomach contents were taken out with a brush and forceps and then dried on filter paper at room temperature. After sorting, stomach items were identified to the lowest taxonomic level feasible (Order-Family) through the aid of microscope later these items were weighed on the balance to calculate their percent frequency of occurrence in the stomach contents following Murton *et al* (1964).

The seasonal fluctuations in the feeding pattern were determined by pooling data among four main seasons occurring in the Indian desert.

4. Results

The examination and analysis of the stomach contents revealed that *R. hardwickei* is primarily an insectivorous species, though some traces of vegetable matter were also observed in summer and monsoon (rainy) seasons (table 1). Fur of the same bat species occurred during summer and monsoon, whereas, it was completely absent during post-monsoon and winter. There were no remains of other animals except insects and spiders.

In winter December to February, Orthoptera (gryllids, house crickets) and Coleoptera (beetles) constitute more than 45% of the total diet. However, Hymenoptera (ants) Lepidoptera (moths), Dictyoptera (Cockroaches) and diptera (flies, mosquitoes) are also preferred in appreciable amounts in decreasing order. Araneida (spiders) were noted in the stomach in moderate proportions during this season.

Table 1. Seasonal fluctuations in stomach contents of *Rhinopoma h. hardwickei*, expressed in percent of total dry mass.

Stomach items	Seasons			
	Winter (Dec.-Feb.) N = 36	Summer (Mar.-June) N = 43	Monsoon (July-Sept.) N = 45	Post-monsoon (Oct.-Nov.) N = 47
Orthoptera				
Gryllidae	22.1	15.1	12.1	10.4
Acrididae	4.0	8.5	5.5	2.9
Isoptera				
Termitidae	...	10.0	28.2	6.8
Dictyoptera	10.9	11.8	1.3	4.7
Lepidoptera				
Noctuidae	4.5	8.1	3.8	4.4
Arctidae	6.1	8.2	10.1	8.2
Unidentified	1.2	...	1.0	0.5
Caterpillars	...	1.2	3.2	1.1
Hymenoptera				
Vespidae	1.2	5.0	5.8	8.8
Formicidae	14.2	4.3	4.5	7.4
Neuroptera				
Mantispidae	2.4	...	1.4	1.3
Diptera				
Chironomidae	3.7	2.2
Culicidae	4.5	1.2	1.3	...
Unidentified	1.0	...	1.1	0.5
Coleoptera				
Scarabaeidae	8.5	4.9	8.3	14.1
Curculionidae	3.4	7.3	3.2	11.5
Carabidae	4.1	2.0	2.0	12.2
Bruchidae	2.0	1.0
Dytiscidae	1.2	4.0
Unidentified	...	1.3	2.2	3.0
Araneida (Spiders)	4.9	1.2
Bat's own fur	...	4.9	3.8	...
Plant parts	...	0.5	1.2	...

Table 2. 'P' values indicating seasonal difference in diet composition of *R. hardwickei*.

Major stomach items	'P' values between successive seasons			
	*W-S N= 79	S-M N= 88	M-PM N= 92	PM-W N= 83
Orthoptera	0.05	0.05	NS	0.05
Isoptera	0.001	0.001	0.001	0.001
Dictyoptera	NS	0.001	0.05	0.001
Lepidoptera	0.01	NS	NS	NS
Hymenoptera	0.05	NS	0.05	NS
Neuroptera	0.001	0.01	NS	NS
Diptera	0.001	NS	0.05	0.001
Coleoptera	0.05	0.05	0.001	0.001
Araneida	0.05	0.01	0.05	0.001
Bat fur	0.001	NS	0.001	NS
Plant parts	0.05	0.05	0.05	NS

*W—Winter, S—Summer, M—Monsoon, PM—Post-Monsoon, NS—Non Significant, N—Total number of bats observed after dissection.

In summer there is an increase in the relative occurrence of Lepidoptera ($P < 0.01$) while Isoptera, (termites, *Odontotermes obessus*, *Anacanthotermes* sp.) which are absent in winter, form about 11% of the total diet (table 2). However, Hymenoptera, Diptera and spiders reduce considerably ($P \pm 0.05$, 0.001 and 0.05 respectively). Preference for Coleoptera ($P < 0.05$), Orthoptera ($P < 0.05$) and Dictyoptera remains more or less same as in winter season.

During monsoon months, when there is abundant insect life in nature, there is a significant rise in the consumption of winged soft-bodied termites (*Microtermes obesii*, *O. obessus*, *Anacanthotermes* sp.), slightly less than three times (28.2%) of the summer season ($P < 0.001$). Relative percent frequency of Coleoptera ($P < 0.01$) and Orthoptera ($P < 0.01$) declines further, while that of Hymenoptera, Diptera and Lepidoptera increases slightly. However, drastic reduction is observed with regard to relative occurrences of Dictyoptera and Spiders in the diet ($P < 0.001$ and 0.01).

In the two months of post-monsoon season, October and November, beetles mainly belonging to families Scarabaeidae (while grubs, *Holotrichia* spp.), Curculionidae and Carabidae constitute major proportion of the diet of bats. Occurrence of ants also increases considerably in the stomachs. Isoptera reduces abruptly, whereas, moderate decline is found regarding consumption of Orthoptera and Lepidoptera. Spiders, bat's own fur and plant parts do not figure at all in this season.

4. Discussion

The small mouse-tailed bat, *R. hardwickei* is primarily an inhabitant of the cave and rocky habitat while about 16% population roosts near or in the midst of human settlement (Advani 1981a). Its roosting habitats have certainly an impact on its feeding behaviour particularly in deciding the composition and seasonal relative occurrence of various insect orders like Diptera, Dictyoptera, Hymenoptera and Orthoptera which are available in and around human environment and Coleoptera (bruchids, scarabaeids, carabids), Isoptera and Lepidoptera which occur in abundance in agro-ecosystems and forested rocky habitat. However, it appears that the feeding habits of this species are also probably a combination of opportunism and selective predation, varying with local ecobiotic conditions such as relative abundance of different kinds of vegetation patterns on which the insect fauna exists. Occurrence of traces of plant parts in the stomachs of bats during summer and monsoon is perhaps due to the remains of undigested gut contents of insects eaten by bats. The presence of orthopterans, caterpillars of Lepidoptera, spiders and some ground beetles suggests that this species also feeds by picking these animals from the ground or other surfaces. Likewise, as observed the drinking behaviour of *R. hardwickei* of skimming over the water surfaces is also very similar to those of allied species *R. microphyllum* (Advani 1981b). However, the requirement of water is also compensated in the desert by the almost exclusive diet of the insects which contain 80–90% water (Robinson 1928). The presence of water beetles (dytiscids : *Lacconectus* sp., *Agabus* sp. *Rhantus* sp.) in the stomachs indicate the ability of bats to swoop over the water surfaces and pick up the most active insects. Regarding composition of food items, *R. hardwickei* markedly differs from the Indian false vampire, *Megaderma lyra lyra* which depends upon an equal proportion of insect and the vertebrate (lizards, fishes, birds etc.) animal diet (Advani 1981c) on an annual basis.

Seasonwise, during winter when temperature falls to about 4–5° C in the Rajasthan desert, the bats are relatively inactive and they thrive upon insects available in their vicinity or home ranges. These include mosquitoes, flies, gryllids, house crickets, cockroaches, ants and beetles, forming major portion of their diet. In this season bats under extreme climatic conditions can also subsist upon their own fat reserves which they accumulate after the monsoon season. During summer and monsoon months preference for termites is quite obvious, as this period coincides with emergence of winged, soft bodies, slow flying termites after the first few showers (From mid June onwards) in Rajasthan. Likewise, in post-monsoon season, occurrence of winged ants and wasps and abundant beetles determine the diet composition of this species. However, the climatological differences among four main seasons, mainly temperature and rainfall variations, govern reproduction, metamorphosis and abundance patterns of various insect orders which act as food for insectivorous bats. These parameters also cause changes in activity and behaviour of bats, as in winter being relatively less active their foraging range is confined to places nearby roosting habitat.

The survival of bats upon some of the most prominent and polyphagous insect species of summer as well as winter crops in Rajasthan desert like *O. obessus*,

M. obesii (termites) ; White grubs (*Holotrichia* spp.) and Cuculionids (Coleoptera) and several grasshopper species, evidently show that this species plays an important role in the ecological balance of the population of these harmful insects in natural crop ecosystem. On the other hand, occurrence of predatory insects like Neuroptera in stomachs, though in low relative percentages, points out towards non-beneficial aspect of feeding ecology of this species.

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References

- Advani R 1981a Bioecological evaluation of the Chiroptera fauna of desert biome of Rajasthan ; *Zeit. angew. Zool.* **68** 281-305
- Advani R 1981b Food and feeding ecology of the Rat-tailed Bat in the Rajasthan desert ; *Acta Theriol.* **26** 269-272
- Advani R 1981c Seasonal fluctuations in the feeding ecology of the Indian false vampire, *Megaderma lyra lyra* (Chiroptera : Megadermatidae) in Rajasthan ; *Zeit. für Saugetierk.* **46** 90-93
- Advani R and Vazirani T G 1981 Studies on ectoparasites of bats of Rajasthan and Gujarat ; *Rec. Zool. Surv. India* Delhi, Occasional Paper No. 22 1-155
- Murton R K, Westwood M J and Isaacson A J 1964 Feeding habits of the Wood pigeon, *Columba palambus*, Stockdove, *Columba cenas* and Turtle dove, *Streptopelia turtur*. *Ibis*, **106** 177-188
- Prakash I 1963 Taxonomic and biological observations on the bats of the Rajasthan Desert ; *Rec. Ind. Mus.* **59** 149-170
- Prakash I, Gupta R K, Jain A P, Rana B D and Dutta B K 1971 Ecological evaluation of rodent populations in the desert biome of Rajasthan; *Mammalia* **35** 384-407
- Robinson W 1928 Water conservation in insects ; *J. Econ. Ent.* **21** 897-902
- Sinha Y P and Advani R 1976 Notes on food and reproduction of some Rajasthan bats ; *Geobios*, **3** 37-40

The annual reproductive cycle of *Achaetobonellia maculata* Fisher (Echiura : Bonellidae)

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Abstract. This study is the first to detail the annual reproductive cycle of any echiuran. Here the annual reproductive cycle of *Achaetobonellia maculata* Fisher is described. Oocytes first appear in the coelomic fluid in late spring or early summer. During fall and winter, gametes production and differentiation continue. Differentiation of gametes lasts four to six months. Spawning occurs in spring. Since the males are the permanent residents in the gonoduct of the female, the fertilization is internal in Bonellidae. Temperature of the sea water probably is the most important exogenous factor controlling the reproductive cycle. Individuals reach sexual maturity when they are one year old.

Keywords. Reproductive cycle ; spawning ; gonoduct ; accessory cells ; oocyte.

1. Introduction

Little is known about the annual reproductive cycle in Echiura. Although some investigators (Hiraiwa and Kawamura 1936 ; Newby 1940) have reported *Urechis caupo* to be fertile throughout the year (with the exception of one or two months in the summer) this appears to be based solely on general observations but no firm data exist.

A general description of reproduction of Echiura has been provided by Gould-Somero (1975), and Singhal and DattaGupta (1982). Gould-Somero (1975) mentioned that the fertilization is internal in Bonellidae, but the males are already permanent residents in the male-sac of the female gonoduct. In other Echiurans fertilization is external but we do not know what (if any) factors ensure simultaneous spawning by males and females in neighbouring burrows. Partially or completely spawned-out *U. caupo* has been collected in late summer (Ricketts and Calvin 1962 ; Gould 1967), and animals will sometimes spawn in the laboratory if the water temperature is raised above 15° C. Therefore, temperature may be a factor. Pilger (1977) studied the annual reproductive cycle in *Listriolobus pelodes*. He found that ovulation lasts three to five months and spawning takes place in spring. Singhal and DattaGupta (1982) reported that oocytes were present

in the coelom of *Achaetobonellia maculata* and *Acanthobonellia vulgaris* for about nine months in a year. This study included a determination of the size at which *A. maculata* becomes sexually mature, the structure of the gonads, the development of the reproductive cells in the coelom and the time, durations and geographical variation in spawning.

2. Materials and methods

A. maculata is a common species of the Pirotan Island, Gulf of Kutch (DattaGupta and Singhal 1978). Since its discovery by Fisher (1953) from the central lagoon of Onotoa, Gilbert Island, the species has not been reported from anywhere except from the aforesaid locality. The population of *A. maculata* from Pirotan Island was studied for its reproductive cycle during 1976 and 1978. Specimens were collected and fixed every month using the method described by Singhal and DattaGupta (1980). Gonads were fixed in Gilson's fluid sectioned at 8μ and stained with Delafield's hematoxylin and eosin.

In *A. maculata* the young oocytes are associated with a complex of accessory cells during the first stage of development. Later they lose these cells and continue to develop without them. Oocytes with accessory cells will be referred to as "stage I" and those without them as "stage II". To determine the cycle, a sample of coelomic fluid was withdrawn with a syringe from each of five females for each month. Twenty oocytes of each stage were measured for their diameter in each female making a total of 100 for each stage per month. The means of stage I and stage II were adjusted according to the percentage contribution of each stage to the coelomic gamete population. This was accomplished by calculating the mean weighted diameter for each month using the formula :

$$PSI_n \bar{X} SI_n + PSII_n \bar{X} SII_n = \text{mean weighted diameter}$$
 where PSI_n and $PSII_n$ are the percentage of stage I and stage II oocytes respectively in the coelom of five female *A. maculata*, during the month n . The values $\bar{X} SI_n$ and $\bar{X} SII_n$ are the mean diameters for the stage I and stage II oocytes during the same month.

As another measure of reproductive periodicity, the concentration of stage I and stage II oocytes within the coelom was determined. To accomplish this, a small sample of coelomic fluid was removed. After fixation and during storage, individual *A. maculata* tend to lose coelomic fluid by diffusion through the body wall and appear deflated although the actual volume it can contain remains unchanged. The net result of this is that the coelomic cells are more concentrated than under normal conditions. To remedy this situation, the individuals were "inflated" with 70% isopropyl alcohol until they reached a subjectively determined uniform tension. Each specimen was shaken to mix the coelomic cells before removal of the sample. The sample then was diluted by an equal volume of 70% isopropyl alcohol and the concentration of each gamete type was determined using a hemocytometer. Ten values were obtained from each of the five females every month from September 1976 to December 1978.

The reproductive cycles were analyzed with several environmental parameters using a multiple regression analysis program. This program is part of the Statistical Analysis System (sas) and was developed by Barr *et al* (1976). Also from

SAS, Backward Elimination and Maximum R^2 Improvement variable selection procedures were used to determine which, if any, of the parameters contribute significantly to the cycle. Coelomic gamete concentration and weighted gamete diameter are handled separately as the dependent variables. The independent variables include DDT, cadmium, organic nitrogen, sulphide, and bottom temperature.

3. Results

The differentiation of gametes consists of three distinct phases. The gametes begin their development while attached to the gonads. They are in the second phase when they break loose and continue their growth floating freely within the coelom. Finally mature gametes are collected and stored in the gonoducts until spawning. The structure of the gonads of *A. maculata* has already been described (Singhal and DattaGupta 1982).

3.1. Size at sexual maturity

The smallest sexually mature female specimen found weighed 2.0 g and measured 25 mm long from mouth to anus. In a sample of 100 female individuals, none weighing less than 2.0 g was sexually mature. Since the male is a permanent resident of the male-sac of the gonoduct of the female, the weight of the female specimen also includes the weight of the male and it cannot be determined as to what is the smallest size and weight of the male for sexual maturity.

3.2. Coelomic oocyte diameters

The mean diameters of stage I and stage II coelomic oocytes are shown in figure 1. Each point represents the mean of 100 measurements and the solid bar equals one standard deviation. The smallest stage I oocytes are present in the coelom during the summer months. These cells are 5–7 μm in diameter. The mean diameter of these oocytes begins to increase during the early fall and by November has reached 22 μm . Since this is a mean value, it does not indicate the upper size limit of stage I oocytes. The actual size of an oocyte when it loses its accessory cells is 40 to 42 μm in live material. A mean diameter of app. 22 μm is maintained until late spring when it begins to decrease. This decrease is due to the transformation of large stage I oocytes into stage II.

Stage II oocytes appear in November at the time when the stage I oocytes first reach their maximum mean diameter. The mean size of stage II oocytes increases through the spring. By June, all of the stage II oocytes have been collected from the coelom by the gonostome and accumulated in the egg-sac of the gonoduct. The actual size of an oocyte when it is removed from the coelom is 60–62 μm stage II oocytes are not present in the coelom again until fall.

Also shown in figure 1 is the weighted average diameter of stage I and stage II oocytes combined. An annual cycle clearly is seen in this representation. Small oocytes first appear in the summer. Most of their growth takes place during the fall and winter months.

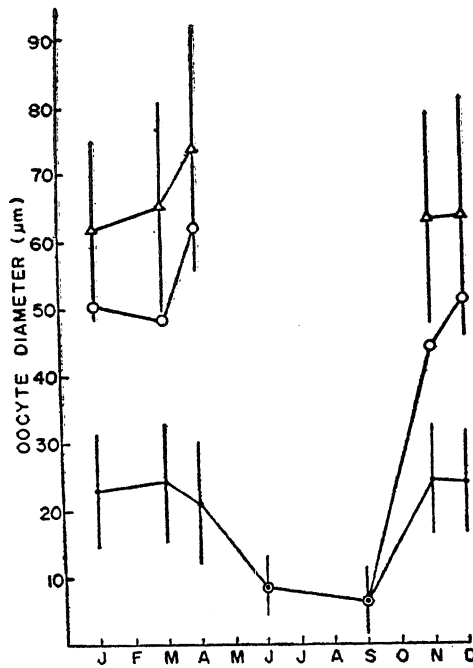


Figure 1. The mean diameter of coelomic oocytes in *A. maculata*. The bar equals ± 1 standard deviation. ● = Stage I oocytes; △ = Stage II oocytes; ○ = weighted mean diameter of stage I and stage II oocytes combined.

The diameters of fixed oocytes differ from those of live oocytes. This was tested by measuring the diameters of 100 live oocytes and 100 fixed oocytes from the same individual and comparing their means. The results show that there is less than a 3% increase in the average diameter after fixation. Since relative values are more important than absolute values, the increase is considered insignificant.

3.3. Coelomic oocyte concentration

Figure 2 indicates the concentration of coelomic oocytes. During spring and first half of the summer stage I oocytes are at their lowest concentration ($2.2-3.5/\text{mm}^3$). In specimens collected in July and August, stage I oocytes concentration begins to increase. By September, the concentration has increased four-fold. By November, stage I oocytes reach their highest concentration and become stage II oocytes by the loss of the accessory cells. As more and more stage I oocytes reach this point their concentration slowly decreases, reaching the lowest level again in summer. Oocytes first appear in November and rapidly reach maximum concentration. From December through spring their concentration declines steadily as they are accumulated in the gonoduct. By June, stage II oocytes are not present in the coelomic phase at all.

These data illustrate the same reproductive cycle as do the oocyte diameter data. During the summer, few coelomic gametes are present. Their number increase through the fall and early winter. During late winter and spring they are collected in the storage organ until spawning. Spawning apparently extended from spring until the end of winter.

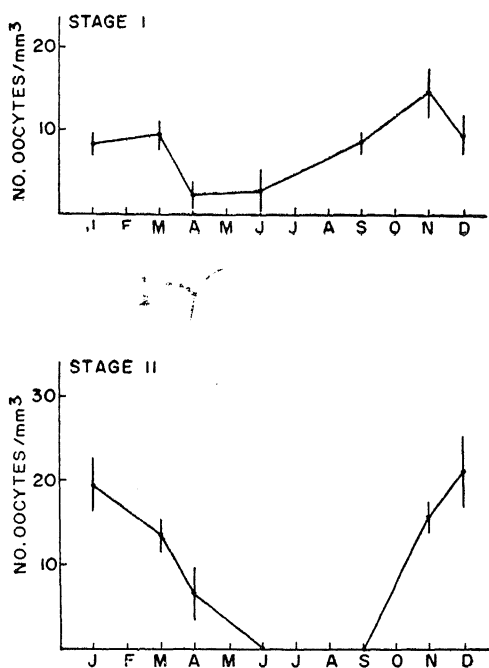


Figure 2. The mean concentration of stage I and stage II coelomic oocytes in *A. maculata*. The bar equals 95% confidence interval.

3.4. Regression analysis of abiotic parameters with the reproductive cycle

By regressing the abiotic data from Pirotan Island (DDT, cadmium, organic nitrogen, sulphide, and bottom temperature) against the weighted mean oocyte diameter, it was determined that the environmental parameters did not account significantly for the variation of oocyte size ($P < 0.05$). However, temperature, nickel and sulphides accounted for a significant amount of the oocyte size variation during the study period ($R^2 = 0.78$, $P < 0.01$). Both Backward Elimination and Maximum R^2 Improvement techniques generated the same three-variable model that accounts for 92% of the variation in oocyte diameter over time ($R^2 = 0.92$, $P < 0.001$). The independent variables selected by these procedures are in order of decreasing importance, bottom water temperature ($P < 0.01$), concentrations of nickel ($P < 0.01$) and sulphides ($P < 0.05$) in the sediment. Adding the remaining independent variables does not significantly improve the predictability of the model.

Regressing all of the independent variables (environmental parameters) against the mean coelomic oocyte concentrations showed no significant contribution ($R^2 = 0.85$, $P < 0.05$). By variable selection methods, however, it was found that two parameters contribute nearly 81% of the variation ($R^2 = 0.79$, $P < 0.01$). These are in order of decreasing importance, the concentrations of sulphide ($P < 0.01$) and DDT ($P < 0.05$). Thus, only a few of the parameters measured are important in determining the number of oocytes produced.

4. Discussion

Unfortunately there is still insufficient information available on the annual reproductive cycles of Echiura to determine how typical the cycle of *A. maculata* is. The smallest sexually mature *A. maculata* encountered in this study is one year old female, 25 mm long, weighing 2.0 g. This is reasonably consistent with the observation of Fisher (1946) who found a 7 mm mature specimen. Baltzer (1931) reported that females of *Bonellia viridis* require two years to reach sexual maturity while the males mature in one or two weeks. *U. caupo* also requires one year to reach sexual maturity.

No studies of annual reproductive cycles in echiurans are available for comparison. However, seasonal gamete production has been reported in the echiurans *Ikedosoma gogshimense* (May and June) (Sawada and Ochi 1962) and *U. unicinctus* (Winter) (Hiraiwa and Kawamura 1936). In direct contrast to this, *U. caupo* produces gametes continuously and contains all oocyte sizes in the coelom simultaneously (Gould-Somero 1975).

The dynamics of oocyte development including the transition from stage I to stage II have been illustrated in diameter frequency polygons. Because *A. maculata* does not produce gametes continuously throughout the year, the frequency of the various oocyte size classes is not proportional to the amount of time the oocyte spends in a particular size class as has been suggested for *U. caupo* (Gould-Somero 1975).

Although the frequency polygons do not provide direct information as to the time course of oogenesis, a rough estimate can be made based on the distribution of the mean diameter of stage I and stage II oocytes over time. The duration of stage I can be estimated by determining the time interval between the onset of increase of stage I mean diameter, which occurs in summer, and the first appearance of stage II oocytes. For instance, at Pirotan Island, after summer the mean diameter of stage I oocyte of *A. maculata* began to increase one month later, stage I oocytes had grown to 22 μ m and had become stage II. Thus, it is predicted that stage I lasts from one to two months.

The period of time from the initial appearance of stage II oocytes in the coelom until they reach their maximum diameter provides an estimate of the duration of this phase of growth. Stage II oocytes appeared in coelom after being absent over the summer and reach their maximum diameter after two months, indicating a two-month period of differentiation. Based on the data available for summer, the duration of stage II differentiation is estimated to be 1½ months ($\pm \frac{1}{2}$ month). These data predict, therefore, that stage II lasts from one to two months.

Combining the estimate for stage I and stage II oocyte differentiation gives a range for the time course of oogenesis of three to five months.

Das (1976) has studied the cytochemical and biochemical processes of oogenesis in *Urechis*. By radioactive labelling he has determined that the duration of the period of oocyte differentiation is 135 days. This closely resembles the estimate for *A. maculata* derived from the reproductive cycle data.

Based on the oocyte diameter data, spawning among *A. maculata* population occurred during spring. The data show that the exact time of spawning and the

length of the period preceding resumption of oocyte growth can vary from year to year (Giese 1959a).

The regression analysis demonstrates that temperature plays an important part in determining the gametogenic cycle. Orton's Rule first proposed by Thorson (1946) states that sea temperature is related to the reproductive cycles of marine organisms. While this is important to many animals, other exogenous and endogenous factors may also play vital roles (Giese 1959b ; Giese and Pearse 1974). Controlled laboratory experiments are necessary to define the environmental components essential for determining any gametogenic cycle (Giese and Pearse 1974).

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References

- Baltzer F 1931 Entwicklungsmechanische Untersuchungen an *Bonellia viridis*. Die Abhängigkeit der Entwicklungsgeschwindigkeit und des Entwicklungsorades der männlichen larvae von der dauer der Russelparasitismus ; *Rev. Suisse Zool.* **38** 361-371
- Barr A J, Goodnight J H, Sall J P and Helwig J T 1976 *A User's Guide to SAS* (Raleigh : Sparks Press) 1-329
- Das N K 1976 Cytochemical and biochemical analysis of development of *Urechis* oocytes ; *Am. Zool.* **16** 345-362
- DattaGupta A K and Singhal R N 1978 Morphology and histology of the males of *Acanthobonellia* ; *Proc. Royal Soc. Edin.* **68** 267-276
- Fisher W K 1946 Echiuroid worms of the North Pacific Ocean ; *Proc. U.S. nat. Mus.* **96** 215-292
- Fisher W K 1953 A new genus of bonellid worms (Echiura) ; *J. Wash. Acad. Sci.* **43** 258-259
- Giese A C 1959a Comparative Physiology ; Annual reproductive cycles of marine invertebrates ; *A Rev. Physiol.* **21** 547-576
- Giese A C 1959b Reproductive cycles of some West Coast Invertebrates. In *Photoperiodism and Related Phenomena in Plants and Animals* ; (ed.) Robert B Withrow (Washington D.C. : Am. Assoc. Adv. Sc.) 1-55
- Giese A C and Pearse J 1974 *Introduction : General principles of reproduction of marine invertebrates* (San Francisco : Academic Press) **1** 501-546
- Gould M C 1967 *Methods in Developmental Biology* (New York : Crowell) 1-163
- Gould-Somero M 1975 *Reproduction in marine invertebrates* (San Francisco : Academic Press Inc.) **3** 201-277
- Hiraiwa Y and Kawamura T 1936 Relation between maturation division and cleavage in artificially activated eggs of *Urechis unicinctus* ; *Biol. Bull.* **70** 344-351
- Newby W W 1940 The embryology of the echiuroid worm *Urechis caupo* ; *Mem. Am. Phil. Soc.* **16** 1-213
- Pilger J F 1977 Biology and gametogenesis of *Listriolobus pelodes* ; Ph.D. Thesis, University of California 1-205
- Ricketts E and Calvin J 1962 *Between pacific tides* (California : Stanford Univ. Press) 1-308

- Singhal R N and DattaGupta A K 1980 Ecological notes on a few Echiuran Animals; *Indian J. Marine Sci.* **9** 139-140
- Singhal R N and DattaGupta A K 1982 Studies on some aspects of reproduction in *Achaetobonellia maculata* Fisher (Echiura : Bonellidae); *N.Z. J. of Zool.* **9** 215-231
- Sawada N and Ochi O 1962 Studies on the fertilization in eggs of the echiuroid, *Ikedosoma gogoshimense* (Ikeda); *Mem. Echime Univ.* **4** 437-444
- Thorson G 1946 Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planktonic larvae of the Sound (Oresund); *Medd. Kommn. Danm. Fisk. Havunders. Plankton* **4** 1-523

Synthesis of 4-methyl (6,7-*b*-tetrahydrobenzofurano) coumarin and its contraception like properties in male rabbits (*Oryctolagus cuniculus*)

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Abstract. Administration of 4-methyl (6,7-*b*-tetrahydrobenzofurano) coumarin, 20 mg/kg/alternate day, for a period of 40 days caused degenerative changes in the testes of male rabbits. Inhibition of spermatogenesis was achieved at primary spermatocyte stage level. Total protein, sialic acid and glycogen contents of the testes, epididymis and seminal vesicle were significantly reduced while the testicular cholesterol was elevated in the 4-methyl coumarin treated animals. Serum cholesterol, phospholipid, triglyceride, NEFA, were elevated. Antispermatic activity of 4-methyl coumarin is discussed.

Keywords. 4-methyl coumarin ; inhibition of spermatogenesis ; sialic acid ; anti-androgenicity.

1. Introduction

Simple aliphatic compounds like triethylene melamine exhibit antifertility properties (Jackson 1964), characterized by damage of spermatogonia and germinal epithelium (Steinberger 1962). Lednicer *et al* (1965) prepared a number of 3,4-diaryl coumarins sterically related to 1,2-diaryl indene and showed that some of these possess antifertility activity.

Marked antifertility activity was also observed in the compounds incorporating triarylethylene and also in 3,4-diphenyl, 1,2,3,4-tetranaphthalene when the hydroxy or alkoxy group was introduced. Mishra and Agrawal (1977) synthesized several new *bis* and *di* (or 4'-coumarynyl-oxyalkanes) coumarins and later tested them for possible antifertility activity.

Realising the importance of benzofuran, coumarin and cyclohexanol derivatives (Tyagi *et al* 1979) as antifertility agents, it was considered worthwhile to design molecule incorporating benzofuran, coumarin and cyclohexanol moieties.

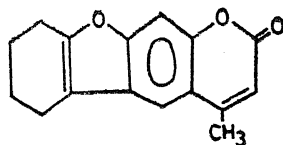
2. Experimental

In this direction 2-halo-cyclohexane was condensed with 7-hydroxy, 4-methyl coumarin. 4-methyl-7-hydroxy coumarin (4.4 g, 0.025 M), 2-bromocyclohexanone (5.31 g; 0.03 M), anhydrous K_2CO_3 (8.0 g) and dry acetone (80.0 ml) were taken in a round bottom flask, fitted with a refluxed condensor. The reaction mixture, after refluxing for 60 hr, was cooled and filtered.

The solvent was distilled off under vacuum. The crude product was crystallized with 95% ethanol. A white crystalline solid compound was obtained. The purity was ascertained by TLC (m.p. 160° C; yield 3.50 g, 55%, R_f 0.89). Its derivative with 2,4-dinitrophenyl hydrazine was prepared (m.p. 169–170° C).

NMR spectrum was obtained in TFA using TMS as internal standard. NMR spectrum indicated the presence of singlet for 3 protons at δ 2.4. In the spectrum complicated pattern for 8 proton in the range of δ 1.1 to 2.1, a broad singlet for a proton at δ 6.2 and 2-protons in the aromatic region (δ 6.75 to 7.45) were observed. The NMR spectrum accounted well for the presence of 14 protons. The presence of a singlet at δ 2.4 was due to the C-methyl ($C-CH_3$) groups attached to position 4 in coumarin system. Methyl group being attached to an aromatic ring and the olifinic bond, conjugated to a carbonyl group alongwith its presence in a pyron ring gave a broad singlet to the down field at δ 2.4. The presence of a broad singlet at δ 6.2 for a proton accounted for the presence of olifinic proton. The presence of only two aromatic protons indicated fusion of cyclohexane ring to the ortho position of -OH group. The presence of 8-protons at much higher field (δ 1.1 to 2.1) indicated the presence of 4-methylene groups and the absence of methane protons at the same time. This clearly indicated the presence of cyclohexane system.

Based on the observations IR/NMR the structure of the compound I was assigned as 4-methyl (6,7-*b*-tetrahydrobenzofurano) coumarin (Tyagi *et al* 1980).



4-methyl (6,7-*b*-tetrahydrobenzofurano) coumarin

3. Material and methods

Twenty healthy adult male rabbits were used in the experiment and were divided into groups as outlined in table 1. Ten rabbits comprising group 2 were treated with 4-methyl (6,7-*b*-tetrahydrobenzofurano) coumarin 20 mg/kg/each alternate day s.c. for 40 days. An equal number of rabbits received the vehicle alone and served as control. After the completion of the final dose of 4-methyl coumarin, rabbits were sacrificed with nembutal anaesthesia. Blood was withdrawn through cardiac puncture and serum analysed.

Table 1. Changes in the weight of testis, epididymis and adrenal glands together with seminiferous tubule and Leydig cell nuclear diameter of rabbit after 4-methyl (6,7-*b*-tetrahydrobenzofurano) coumarin treatment.

	Body weight (kg)	Testes	Epididymis mg/kg	Adrenal	Seminiferous tubule dia- meter (μ m)	Leydig cell nuclear diameter (μ m)
Control (10)	1.4 \pm 0.3	1931 \pm 108	780 \pm 64	210 \pm 35	175 \pm 2.4	6.00 \pm 0.17
4-methyl coumarin (10)	1.3 \pm 0.2*	1336 \pm 146*	470 \pm 43*	278 \pm 26†	114 \pm 1.0**	5.16 \pm 0.148*

4-methyl coumarin *versus* control: ** $P < 0.001$ * $P < 0.02$ †NS (Not significant); All figures \pm S.E.M. Figures in parenthesis represent the number of animals examined.

Final body weight of each animal from both groups were recorded. Testes, epididymis, seminal vesicle and adrenal glands were dissected free of fat. Right testis and epididymis were fixed in Bouin's fluid. 6 μ m sections were prepared and stained with haematoxylin and eosin. Left testis, epididymis, seminal vesicle and adrenal glands were frozen and the total protein, sialic acid, testicular cholesterol, glycogen, acid phosphatase and adrenal ascorbic acid were later determined (Lowry *et al* 1951; Warren 1959; Montgomery 1957; Fiske and Subbarow 1925; Roe and Kuether 1943). Quantitative estimation of cholesterol was made according to the Libermann-Burchard method (Oser 1965). Serum was analysed for cholesterol, phospholipids, triglyceride, non-esterified free fatty acid and serum proteins (Varley 1969). The transaminase enzyme activity (SGPT) was determined according to Mohun and Cook (1957).

One hundred seminiferous tubules appearing circular in sections were traced with camera lucida at $\times 80$. Two perpendicular diameters of each group tracing were measured and expressed in terms of mean tubular diameters. Student's 't' test was applied for comparing means. The measurements of the diameters of 100 Leydig cell nuclei were carried out from the sections of testes with camera lucida drawings at $\times 800$.

The LD₅₀ of 4-methyl (6,7-*b*-tetrahydrobenzofurano) coumarin worked out in white albino rat comes out to be 200 mg/kg.

Dizziness and paralytic conditions were the main symptoms observed.

4. Results

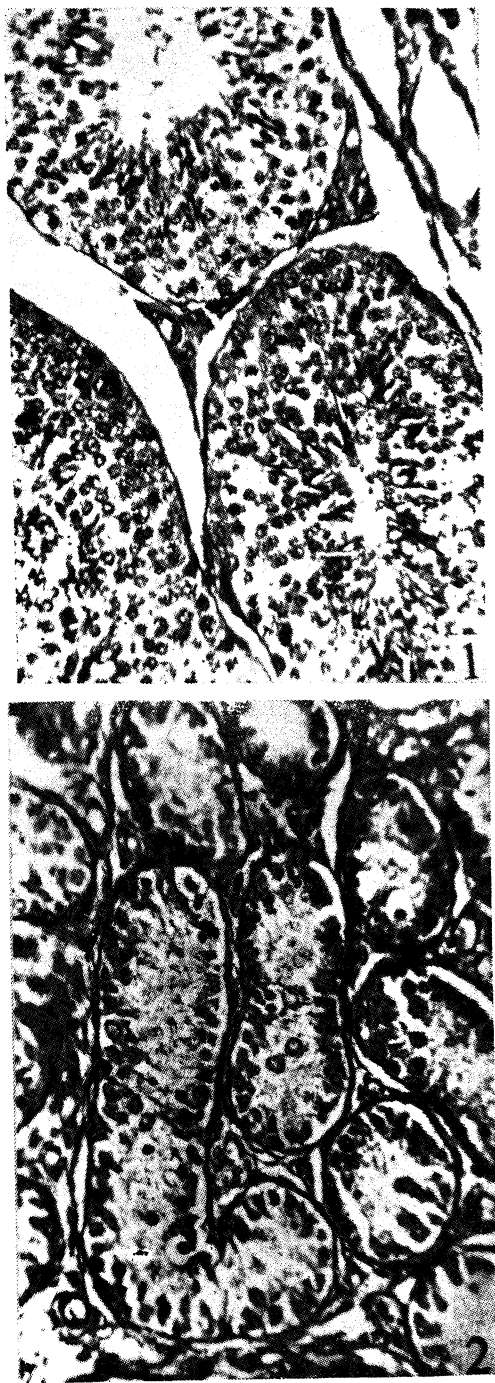
4.1. Body weight and organ weight (table 1)

The body weight of the rabbits treated with 4-methyl coumarin was insignificantly reduced. The testicular weight and epididymal weight exhibit significant reduction in the 4-methyl coumarin-treated animals when compared with controls.

Table 2. Changes in protein, sialic acid, glycogen, cholesterol and adrenal ascorbic acid content of testis, epididymis and seminal vesicle of rabbits after 4-methyl (6,7-*b*-tetrahydrobenzofurano) coumarin treatment.

	Protein			Sialic Acid			Acid Phosphatase			Glycogen		Choles- terol		Ascorbio acid adrenal	
	T	E	SV	T	E	SV	T	E	SV	T	T	T	T	mg/ μ g	mg/ μ g
	μ g/mg														
Control	230 \pm 18	179 \pm 21	177 \pm 6	5.6 \pm 0.3	4.8 \pm 0.2	6.7 \pm 0.3	2.68 \pm 0.3	2.24 \pm 0.3	2.6 \pm 0.3	2.1 \pm 0.3	3.6 \pm 0.6	5.04 \pm 0.51			
4-methylcoumarin	110 \pm 4*	110 \pm 7†	119 \pm 5*	3.8 \pm 0.1*	3.4 \pm 0.1*	3.9 \pm 0.2*	1.63 \pm 0.2*	1.33 \pm 0.2*	1.5 \pm 0.1*	0.9 \pm 0.1†	7.2 \pm 0.5*	3.5 \pm 0.1†			

T = Testis ; E = Epididymis ; SV = Seminal vesicle ; 4-Methyl coumarin *versus* control ; * $P < 0.01$ † $P < 0.05$ All figures \pm SEM
Biochemical estimations : Means of six determinations.



Figures 1-2. 1. Testis of a control rabbit showing various stages of spermatogenesis $\times 160$ HE. 2. After 4-methyl coumarin treatment. Note the loss of various cell stages $\times 160$ HE.

4.2. Histological changes

4.2a. *Testes* : In the rabbits treated with 4-methyl coumarin, the seminiferous tubule diameter and Leydig cell nuclear diameter decreased significantly (table 1). Spermatogenesis was arrested at primary spermatocyte stage. The changes consisted of loss of spermatids and spermatozoa (figures 1, 2). Sertoli cells were normal.

4.2b. *Epididymis* : Histological examination of the epididymis of 4-methyl coumarin-treated rabbits showed that the epithelium was normal and the lumen of caput epididymis was filled with debris. Cauda epididymis and ductus deferens were devoid of spermatozoa.

4.3. Biochemical changes

4.3a. *Protein* : The total protein contents of testis, epididymis and seminal vesicle were significantly lower in the rabbits treated with 4-methyl coumarin in comparison with controls (table 2).

4.3b. *Sialic acid* : The level of sialic acid was significantly decreased in the testis, epididymis and seminal vesicle of 4-methyl coumarin-treated rabbits (table 2).

4.3c. *Acid phosphatase* : Acid phosphatase enzyme activity of the testis, epididymis and seminal vesicle was reduced significantly after 4-methyl coumarin treatment (table 2).

4.3d. *Glycogen* : The glycogen level of testes decreased significantly (table 2).

4.3e. *Cholesterol* : The total cholesterol of testis increased in treated animals (table 2).

4.3f. *Ascorbic acid* : The ascorbic acid contents of adrenal glands were low (table 2).

4.3g. *Serum analysis* : The decrease in serum protein of coumarin-treated animals was highly significant ($P < 0.01$). No significant change was observed

Table 3. Serum analysis of rabbit after 4-methyl (6,7-*b*-tetrahydrobenzofurano) coumarin treatment.

	Protein	Cholesterol	Phospholipid (mg/100 m)	Triglyceride	NEFA mEq/L	SGPT Reitman Frankel units
Control	11420 ± 180	121.4 ± 10.2	130 ± 7.24	73 ± 4	0.244 ± 0.014	99 ± 10
4-methyl- coumarin	8030 ± 213*	152 ± 12†	176 ± 9**	116 ± 3*	0.370 ± 0.02*	109 ± 23†

4-methyl coumarin *versus* control : * $P < 0.01$ ** $P < 0.05$ † $P < NS$ (Not significant).
All figures ± SEM. Biochemical estimations : Means of six determinations,

in pyruvate transaminase activity, however an increase was recorded in the total cholesterol, phospholipid, triglyceride and non-esterified fatty acids (table 3).

5. Discussion

Compounds which suppress spermatogenesis include many chemical classes and modes of action (Jackson 1970). Little information is available concerning structure activity relationships and metabolism of these interesting compounds.

After 40 days of 4-methyl (6,7-*b*-tetrahydrobenzofurano) coumarin treatment resulted in disappearance of mature sperms and spermatids. These results are similar as observed after ethylene dimethane sulphonate (Jackson 1970), nitrofurantoin and α -chlorohydrin (Patanelli 1975). Decrease level of protein in the testes, epididymis and seminal vesicle of rabbits treated with 4-methyl (6,7-*b*-tetrahydrobenzofurano) coumarin suggest an inhibition of spermatogenesis and suppressed Leydig cell function. Podesta *et al* (1975) describe a relationship between the androgen sensitivity and protein synthesis, contents and concentration, in the epididymis. A decrease in the level of protein of epididymis reflects the antiandrogenic nature of the compound. Significant decrease in glycogen may affect protein synthesis and thus subsequently inhibit spermatogenesis.

Reduced acid phosphatase enzyme/sialic acid activity confirms the inhibitory role of 4-methyl coumarin on spermatogenesis in rabbit. Blackshaw and Massey (1978) showed that the total and free biochemical acid phosphatase decreased during cryptorchidism. Peyre and Laporte (1966), Rajalakshmi and Prasad (1968) reported a fall in the sialic acid contents of cryptorchid testes/epididymis of castrated rats and intact langur monkeys (Braz *et al* 1979).

A significant increase in testicular/serum cholesterol after 4-methyl coumarin treatment have been considered physiologically important, since testicular cholesterol derived from blood cholesterol is used for testosterone production (Anderson and Dietschy 1977) and is the primary substrate for androgen biosynthesis (Dorfman *et al* 1963; Eik-Nes and Kekre 1963).

Serum protein was reduced while cholesterol was elevated. The phospholipids, triglycerides and non-esterified fatty acids were also increased in the rabbits following 4-methyl (6,7-*b*-tetrahydrobenzofurano) coumarin treatment, reflects an interference in the lipid metabolism. However, more work is in progress for the reversible action of the compound and shall be reported elsewhere.

Acknowledgements

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References

- Anderson J M and Dietschy J M 1977 Regulation of steroid synthesis in 15 tissues of rat. II. Role of rat and human high and low density plasma lipoproteins and of rat chylomicron remnants; *J. Biol. Chem.* **252** 3652-3659

- Blackshaw A N and Massey P F 1978 The effect of cryptorchidism on the quantitative histology, histochemistry and hydrolytic enzyme activity of the rat testis ; *Aust. J. Biol. Sci.* **31** 53-64
- Braz I, Shandilya L N and Ramaswami L S 1979 Effect of α -chlorohydrin on the male reproductive organs of the Indian Langur (*Presbytis entellus entellus* Dufresne) ; *Andrologia* **8** 290-296
- Dorfman R I, Forchielli E and Gut M 1963 Androgen biosynthesis and related studies ; *Recent Prog. Horm. Res.* **19** 251-273
- Eik-Nes K B and Kekre M 1963 Metabolism *in vivo* of steroids by the Canine testis ; *Biochem. Biophys. Acta* **78** 449-456
- Fiske C M and Subbarow Y 1925 Colorimetric determination of phosphorus ; *J. Biochem.* **66** 375-400
- Jackson H 1964 The effect of alkylating agents on fertility ; *Bri. Med. Bull.* **20** 107-114
- Jackson H 1970 Antispermato-genic agents ; *Bri. Med. Bull.* **26** 79-86
- Lednicer D, Babeock J C, Marlatt P E, Lyster S C and Duncan G W 1965 Mammalian anti-fertility agents. I. Derivatives of 2-3 diphenylindene ; *J. Med. Chem.* **8** 52-57
- Lowry O H, Rosenbrough M J, Farr A L and Randall R J 1951 Protein measurement with the folin phenol reagent ; *J. Biol. Chem.* **193** 265-275
- Mishra V S and Agrawal S 1977 Synthesis of possible antifertility compounds. Synthesis of *bis* and *di* derivatives of coumarins ; *J. Indian Chem. Soc.* **L IV12** 1124-1126
- Mohun A F and Cook I J Y 1957 Simple method for measuring serum levels of glutamic oxaloacetic and glutamic pyruvic transaminases in routine laboratory ; *J. Clin. Path.* **10** 349-399
- Montgomery R 1957 Determination of glycogen ; *Arch Biochem.* **67** 378-379
- Oser B L 1965 In Hawk's *Physiological chemistry* 14th ed. (New York : McGraw-Hill) p. 246
- Patanelli D J 1975 Suppression of fertility in the male. In *Handbook of physiology* edited by D W Hamilton, R O Greep, Sect 7 : *Endocrinology*, Vol. 5 : Male Reproductive system (Washington D C : American Physiological Society) p. 245
- Peyre A and Laporte P 1966 Action de la testosterone et de l'oestradiol sur les sialo proteines de la queue de l'epididyme de rat impubere castré ; *C R Seanc. Soc. Biol.* **160** 2178-2180
- Podesta E J, Calendra R S, Rivarola M A and Blaquier J A 1975 The effect of castration and testosterone replacement on specific protein and androgen levels of rat epididymis ; *Endocrinology* **97** 399-405
- Rajalakshmi M and Prasad M R N 1968 Changes in the sialic acid content of the accessory glands ; *J. Endocrinol.* **41** 471-476
- Roe J H and Kuether C A 1943 The determination of ascorbic acid in whole blood and urine through the 2,4-dinitro phenyl hydrazine derivative of dehydro ascorbic acid ; *J Biol. Chem.* **147** 399-407
- Steinberger E 1962 A quantitative study of the effect of an alkylating agent (Triethylene meta-mine) on the seminiferous epithelium of rats ; *J. Reprod. Fertil.* **3** 250-259
- Tyagi A, Joshi B C, Kumar S and Dixit V P 1979 Spermatogenic activity of cyclohexanol in gerbil (*Meriones hurrianae* Jerdon) and House rat (*Rattus rattus Rufescens*) ; *Indian J. Exp. Biol.* **17** 1305-1307
- Tyagi A, Dixit V P and Joshi B C 1980 New novel non-steroidal antifertility agents ; *Naturwissenschaften* **67** 104
- Varley H 1969 *Practical clinical biochemistry*, 4th ed. (London : White Friers Press Ltd.) p. 126
- Warren L 1959 The thiobarbituric acid assay of sialic acids : *J. Biol. Chem.* **234** 1971-1975

Cellular sites of steroid synthesis in the oviparous teleost fish (*Cyprinus carpio* L.) : A histochemical study

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Abstract. Histochemical techniques for lipids were employed to study the steroid synthesizing cellular sites in the ovary of teleost fish (*Cyprinus carpio* L.). The cellular sites of steroid biosynthesis appear to be the ovulating corpora lutea, interstitial cells, and special thecal cells of developing follicle. They possess the cytological and histochemical features of well-established steroid gland cells. The functional significance of histochemical changes in the granulosa cells of post-ovulatory follicles in the teleost ovary has been discussed in the light of recent researches on corresponding cells in the ovaries of other vertebrates. The corpora atretica are merely the large yolky eggs in the process of their degeneration and resorption.

Keywords. Cellular sites ; steroid synthesis ; histochemistry ; lipids ; ovary ; teleost.

1. Introduction

Histochemical techniques mainly for Δ^5 - 3β -hydroxysteroid dehydrogenase (3β -HSDH) and electron microscopy have been employed for the study of cellular sites of steroid synthesis in the ovaries of some teleosts (see Bara 1965 ; Guraya 1976, 1978a, 1979 ; Nagahama *et al* 1978 ; Kagawa *et al* 1981). However, the precise roles of atretic yolky eggs (corpora atretica or corpora lutea of atresia), postovulatory follicles (corpora lutea of ovulation) and the scarcely developed stroma, together with its interstitial (or thecal gland) cells, in relation to steroid biosynthesis, are still not known. In most of the previous studies carried out with the routine histological techniques the corpora atretica have been claimed to be the main site of steroid biosynthesis in the teleost ovary (Ball 1960 ; Chan *et al* 1967). This opinion was not shared by others (Dodd 1960 ; Polder 1964 ; Guraya *et al* 1975, 1977). No any attempt has been made so far to study the nature of lipid changes during the involution of postovulatory follicles or corpora lutea in teleosts (see Guraya 1979 ; Kagawa *et al* 1981). This study using histochemical techniques for

lipids describes the lipid changes of follicles, postovulatory follicles, corpora lutea of ovulation, corpora atretica or preovulatory corpora lutea and interstitial (thecal gland) cells in the ovary of the scale carp, *Cyprinus carpio* L.

2. Materials and methods

The ovaries of oviparous teleost fish (scale carp *Cyprinus carpio* Linn.) were used. The fish were obtained from the fishery pond of the Punjab Agricultural University, Ludhiana. This fish usually breeds in the months of February and March in the Punjab waters. During the breeding season, two mature males and a female were put in the *hapa*. The spawning usually occurred in the early hours of the morning. Weeds were also added in the *hapa* for the attachment of eggs. The recovery of eggs from *hapa* was the criterion for ovulation. After spawning, the ovary was removed and transferred into physiological saline solution. The ovarian material was collected at 12 hr intervals from different females. It was also collected after an interval of one day. Thereafter, the material was collected on alternate days. These time intervals were taken into account from the time of spawning. After washing off the blood in physiological saline the ovary was immediately fixed in freshly prepared fixing fluids. The details of histochemical techniques used were the same as those reported previously (Guraya 1968).

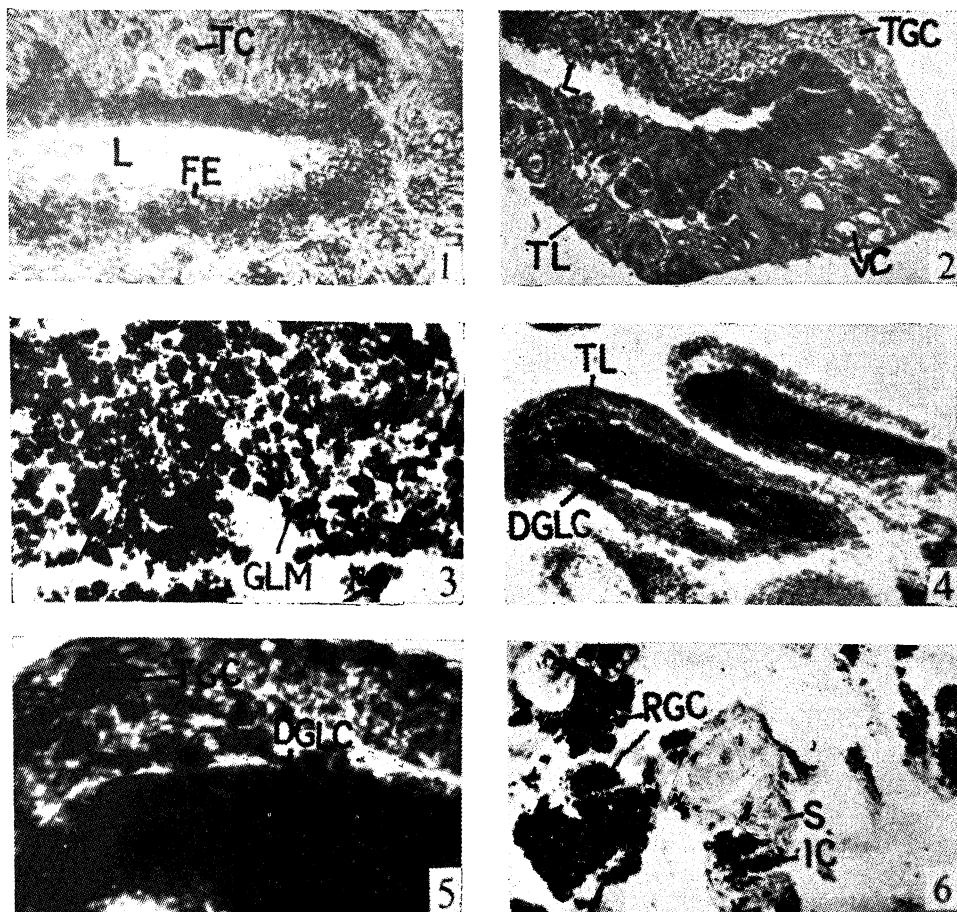
3. Observations and discussion

The vascularized thecal layer of follicle during the preovulatory period consists of fibroblasts and does not show any appreciable development of sudanophilic lipids-containing cells which could easily be demonstrated with the light microscope. The distribution and histochemical nature of lipids in the follicular epithelium are the same as those reported for the teleost *Channa* (Guraya 1976, 1978a). The cytoplasm of follicle (or granulosa) cells shows some sparsely scattered, deeply sudanophilic lipid droplets which give positive reactions for phospholipids, no Schultz-positive substances (cholesterol and/or its esters) are observed.

The follicular epithelium of the developing follicle in the ovaries of some teleosts may show a positive 3β -HSDH activity which is also accompanied by the presence of enzymes of the citric acid and the pentose-phosphate cycles (Guraya 1976, 1978a, 1979). The two enzymes 3β - and 17β -HSDH are not only present in the granulosa cells but also in the cortical cytoplasm during the last phases of follicle growth in the fish ovary. It is still not known whether the presence of these enzyme systems indicates synthesis and secretion of steroid hormones by the granulosa cells of developing follicle *in vivo*, or simply indicates their potentialities for steroidogenesis.

3.1. Postovulatory follicles

The granulosa cells after ovulation develop sudanophilic lipid droplets of variable size during different stages of evolution and involution of postovulatory follicles



Figures 1-6. 1. Portion of postovulatory follicle in stage I showing sudanophilic lipids in the follicular epithelium (FE) and some thecal gland cells (tc) of thecal layer. Lumen (L) is seen. $\times 400$. 2. Portion of postovulatory follicle in stage 2 showing sudanophilic lipids in granulosa luteal cells and thecal gland cells (TGC). Thecal layer (TL) shows some vacuolated cells (vc). Lumen (L) is reduced. $\times 400$. 3. Portion of postovulatory follicle in stage 5, showing heavy accumulation of sudanophilic lipid droplets in the granulosa luteal cell mass (GLM). The cells have separated from each other. $\times 400$. 4. Postovulatory follicles in stage 6, showing heavy accumulation of sudanophilic lipids in degenerated granulosa luteal cells (DGLC). The amount of sudanophilic lipids has also increased in the thecal layer (TL). $\times 100$. 5. Higher magnification of portion of postovulatory follicle shown in figure 4 showing accumulation of sudanophilic lipids in degenerated granulosa luteal cells (DGLC). The thecal layer also shows cells (TGC) filled with sudanophilic lipids. $\times 400$. 6. A portion of ovary showing sudanophilic lipids in residual granulosa luteal cells (RGC) and interstitial cells (ic) distributed in the stroma (s). The latter does not show such lipids. $\times 100$.

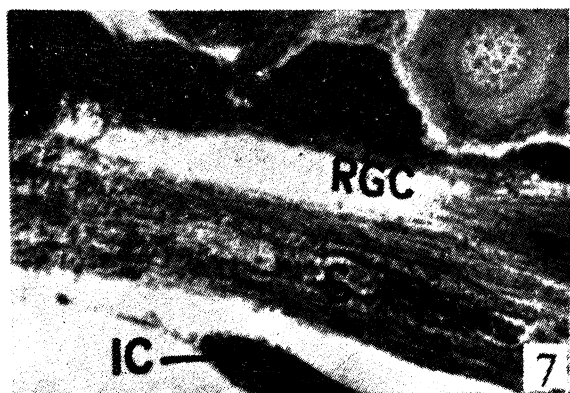


Figure 7. A portion of ovary showing sudanophilic lipids in residual granulosa luteal cells (RGC) and interstitial cells (IC). Stroma proper (s) does not show such lipids. $\times 400$.

(figures 1-7), which consist of first phospholipids, then phospholipids and triglycerides, and finally triglycerides, cholesterol and/or its esters and some phospholipids (table 1). Besides the lipid droplets, they also develop diffusely distributed sudanophilic lipids (lipoproteins) in their cytoplasm (figures 1, 2). Similar lipid droplets and diffuse lipoproteins are also developed during the transformation of granulosa cells into the luteal cells in other vertebrates (see Guraya 1976). Diffusely distributed lipoproteins apparently derive from the membranes of smooth reticulum described for the granulosa cells of postovulatory follicles in teleosts and other vertebrates (Hoar and Nagahama 1978 ; Nagahama *et al* 1976, 1978 ; Guraya 1976, 1979 ; Kagawa *et al* 1981).

The thecal layer of postovulatory follicle in the present fish shows some hypertrophied cells with sudanophilic lipid droplets and diffuse lipoproteins (figures 1, 2) which correspond to the special thecal cells in the spent follicles of other teleosts (Nagahama *et al* 1978 ; Kagawa *et al* 1981). These special thecal cells give a positive reaction for $\beta\beta$ -HSDH and show ultrastructural features of steroid gland cells such as greatly developed agranular endoplasmic reticulum and numerous large mitochondria with tubular cristae (see Nagahama *et al* 1978 ; Kagawa *et al* 1981).

Generally when lipid droplets are abundantly present in the steroid gland cells, storage is taking place and when the amount is less, the steroid hormone is being released (Guraya 1976, 1978a, b, 1979). According to this concept, the granulosa cells in stages 1, 2, 3, which contain a few lipid droplets of the postovulatory follicles (table 1), may be functioning in the secretion of some steroid hormone (see also Guraya 1976, 1979). This suggestion is also supported by the fact that the granulosa cells during these stages show organelles and enzyme activities related to steroid biosynthesis (see Guraya 1976, 1979 ; Hoar and Nagahama 1978 ; Nagahama *et al* 1976, 1978 ; Kagawa *et al* 1981).

The degenerating granulosa cells in stages 4, 5 and 6 of postovulatory follicle (table 1) apparently function in the storage of hormone precursor, as supported by the accumulation of highly sudanophilic, cholesterol-positive lipid droplets (figures 3-7) which constitute the precursor material stored within the steroid gland cells (Guraya 1976, 1978b, 1979). The regression of steroidogenesis during the later life of postovulatory follicle in teleost ovary is also supported by the gradual disappearance of its enzyme activities and alterations in organelles related to steroidogenesis (Lambert and van Oordt 1974 ; Guraya 1976, 1979 ; Nagahama *et al* 1976, 1978). The minimum enzyme $\beta\beta$ -HSDH activity is reached between the third and fourth day after spawning in the zebra fish (Lambert and van Oordt 1974), indicating a short functional life of postovulatory follicle in the oviparous teleosts (Guraya 1976, 1979) as also supported by the results of the present study.

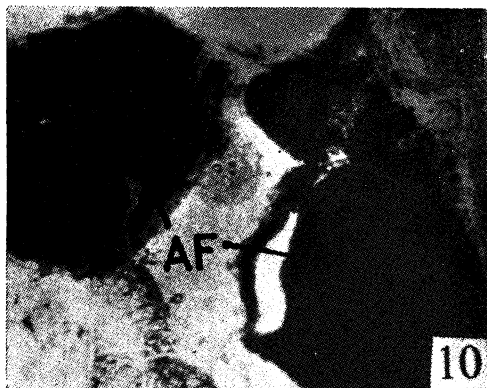
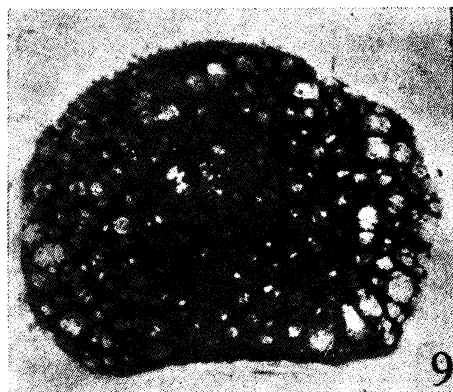
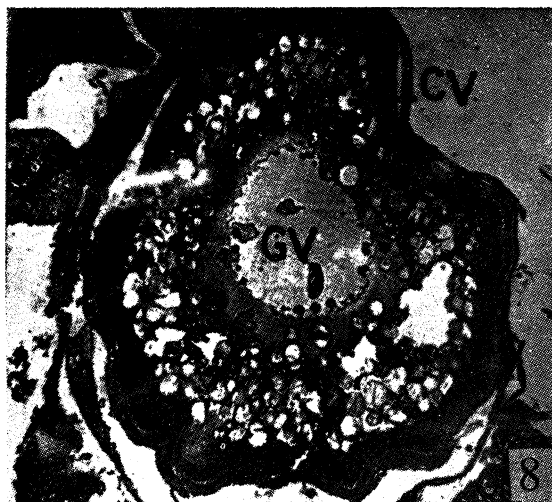
3.2. *Corpora atretica (or preovulatory corpora lutea)*

The first change, which occurs during the atresia of ripe yolky eggs, is the development of large sudanophobic vacuoles in the region of cortical vacuoles (figures 8, 9). As the atresia advances, highly sudanophilic fatty yolk elements coalesce to form

Table 1. Summary of lipid changes in the granulosa and thecal cells during successive stages of postovulatory follicle in the fish *Cyprinus carpio*.

No	Fixation	Reference	Cell types	Diffuse lipids	Lipid droplets						Remarks
					Stage 1	Stage 2	Stage 3	Stage 4	Stages 5, 6		
sk	FCA + PC	After Baker 1946, 1956 (as cited in Pearse 1968)	GC	++	++ to ++	+++	+++	+++	+++	Showing the presence of diffuse lipid and discrete lipid droplets in granulosa cells (GC) and hypertrophied thecal cells at different stages of postovulatory follicle	
	TC		+	+++	+++	+++	+++	+++			
and	WB + PE	Cited in Pearse 1968	GC	—	—	—	—	—	—	Revealing the increasing development of neutral fats in the lipid droplets of granulosa and thecal cells at different stages.	
	TC		—	—	—	—	—	—			
	FCA + PC	After Cain 1947 (cited in Pearse 1968)	GC	+ blue	P +++	P +++	P +++	P +++	P +++	Revealing the increasing development of triglycerides in lipid droplets during the successive stages	
	TC		+ blue	P +	P ++	P ++	P ++	P ++			
AH)	FCA + PC	As cited in Pearse 1968	GC	—	++	++	++	++	++	Revealing the presence of phospholipid in the lipid droplets of granulosa and thecal cells	
	GC		+	+	+	+	+	+			
	FCA + PC	Baker 1946	CC	—	++	++	++	++	++	Revealing the accumulation of cholesterol and/or esters in granulosa luteal cells in stages 4, 5, 6	
	WB + PE		(cited in Pearse 1968)	TC	—	++	++	++	++		++
FCA + PC	Pearse 1968	TC	—	—	—	—	++ to ++	++	++	Revealing the accumulation of cholesterol and/or esters in granulosa luteal cells in stages 4, 5, 6	
		GC	—	—	—	—	++	++	++		

Abbreviations: *, after treatment with; FCA + PC, formaldehyde-calcium and postchromed in dichromate-calcium; P, pink; WB + PE, Weak Bouin followed by yridine extraction; +, weak reaction; ++, moderate reaction; ++++, strong reaction; ++ to ++, very strong reaction; —, negative.



Figures 8–10. 8. Yolk eggs in its early stage of atresia, showing sudanophobic vacuoles at the periphery (cv). Follicular epithelium and zona pellucida are still intact. Germinal vesicle (gv) is also seen. $\times 400$. 9. Corpus atreticum in stage 3 showing accumulation of sudanophilic lipids in granulosa cells. The yolk has been ingested by the granulosa cells. $\times 400$. 10. Showing degenerated corpora (AF) in stage 4, filled with sudanophilic lipids. $\times 400$.

highly sudanophilic masses (figures 8, 9, 10). The yolky contents of atretic follicles are gradually digested and removed by the granulosa cells which, at the same time, store lipids consisting of triglycerides and some phospholipids. Similar lipids also accumulate in the granulosa cells of atretic follicles in other vertebrates (Guraya 1976). In their later stages of resorption, atretic yolky eggs in the present teleost also store cholesterol and/or its esters. The corpora atretica or 'pre-ovulatory, corpora lutea of lower vertebrates are usually believed to secrete steroid hormones (see references in Browning 1973). But Guraya (1976) believes that they are merely the large eggs in the process of their resorption as also observed in this study. The various enzyme cytochemical investigations have also shown that the corpora atretica of teleost ovary are merely the large yolky eggs in the process of degeneration and resorption (Guraya 1976, 1979). The dense lipid and cholesterol accumulations demonstrated in the atretic yolky eggs of the present carp may be due to degenerative changes.

3.3. Interstitial cells

The interstitial cells in the ovaries of present teleost occur singly or in groups in the stroma (figures 6, 7). Some of them appear to derive from the persisting hypertrophied thecal gland cells of postovulatory follicles as well as from their residual granulosa cells (figures 6, 7). They are filled with sudanophilic lipid droplets consisting of triglycerides, cholesterol and/or its esters and some phospholipids. The thecal gland cells, described by Nicholls and Maple (1972) in the wall of the cichlid fish follicles, may be the interstitial (or thecal) gland cells having the cytological features of steroid-secreting cells. These special thecal cells are believed to be the only cells responsible for steroid production in the thecal layer of salmonid fishes (Nagahama *et al* 1978 ; van den Hark and Peute 1979). Kagawa *et al* (1981) have also identified special thecal cells in the wall of preovulatory follicle of *S. leucomaenis*, which show mitochondria with tubular cristae and both agranular and granular forms of endoplasmic reticulum, the latter being more prominent. The results of these various studies have indicated that interstitial cells form the important component of the teleost ovary. With the growth of vitellogenic follicles, they get sparsely distributed in their walls and then are called as special thecal cells or thecal gland cells. The presence of interstitial cells has also been demonstrated in the ovaries of different vertebrates (see Guraya 1976). The ovarian interstitial cells of these vertebrates possess the cytological and histochemical features of well-established steroid gland cells (Guraya 1976). It can also be presumed that the interstitial (or thecal gland) cells of present teleost ovary are steroid secretors as they contain cholesterol-positive lipids in their cytoplasm under certain physiological situations, and are associated with blood vessels. This suggestion is further supported by the presence of 3β -HSDH activity indicative of steroidogenesis in the ovarian interstitial (or thecal gland) cells of teleosts (Lambert and van Oordt 1974 ; Guraya 1976, 1978a, 1979) as well as by the various electron microscope studies (see Nagahama *et al* 1978 ; Kagawa *et al* 1981). In the ovaries of teleosts investigated by Lambert and van Oordt

(1974), the 3β -HSDH positive interstitial cells are mainly distributed in the stroma, as well as against the follicle wall. They show conspicuous fluctuations in their distribution and enzyme contents with the ovarian cycle. The physiological significance of the interstitial cells remains doubtful as in the Swordtail where they show clear cytochemical indications of steroid metabolism, but in the zebra fish they lack any glucose-6-phosphate dehydrogenase activity (Lambert and van Oordt 1974). Kagawa *et al* (1981) have attributed the secretion of high progesterone levels to the special thecal cells of postovulatory follicle in the white-spotted char rather than to its granulosa luteal cells.

References

- Ball J N 1960 Reproduction in female bony fishes; *Symp. Zool. Soc. London* **1** 105-135
- Bara G 1965 Histochemical localization of Δ^5 - 3β -hydroxysteroid dehydrogenase in the ovaries of a teleost fish *Scomber scomber* L.; *Gen. Comp. Endocrinol.* **5** 284-296
- Browning H C 1973 The evolutionary history of the corpus luteum; *Biol. Reprod.* **8** 128-157
- Chan S T H, Wright A and Phillips J G 1967 The atretic structures in the gonads of the rice-field eel (*Monopterus albus*) during natural sex reversal; *J. Zool. (Lond.)* **153** 527-539
- Dodd J M 1960 Gonadal and gonadotrophic hormones. In *Marshall's physiology of Reproduction* (ed.) A S Parkes (London: Longmans, Green and Co.) Vol. I Part II pp. 417-558
- Guraya S S 1968 Histochemical study of granulosa (follicular cells) in the preovulatory and postovulatory follicles of amphibian ovary; *Gen. Comp. Endocrinol.* **10** 138-146
- Guraya S S 1976 Recent advances in the morphology, histochemistry and biochemistry of steroid-synthesizing cellular sites in the non-mammalian vertebrate ovary; *Inter. Rev. Cytol.* **44** 365-409
- Guraya S S 1978a Maturation of the follicular wall of non-mammalian vertebrates. In *The vertebrate ovary* (ed.) R E Jones (New York: Plenum Press) pp. 261-329
- Guraya S S 1978b Recent advances in the morphology, histochemistry, biochemistry and physiology of interstitial gland cells of mammalian ovary; *Int. Rev. Cytol.* **55** 171-245
- Guraya S S 1979 Recent advances in the morphology and histochemistry of steroid-synthesizing cellular sites in the gonads of fish; *Proc. Indian natn. Sci. Acad.* **B45** 432-461
- Guraya S S, Kaur R and Saxena P K 1975 Morphology of ovarian changes during the reproductive cycle of fish, *Mystus tengara* (Ham.); *Acta Anat.* **91** 222-260
- Guraya S S, Toor H S and Kumar S 1977 Morphology of ovarian changes during the reproductive cycle of the fish *Cyprinus carpio communis* (Linn.); *Zool. Baitrage* **23** 405-407
- Hark R van den and Peute J 1979 Cyclic changes in the ovary of the rainbow trout, *Salmo gairdneri*, with special reference to sites of steroidogenesis; *Cell Tissue Res.* **199** 289-306
- Hoar W S and Nagahama Y 1978 The cellular sources of sex steroids in teleost gonads; *Ann. Biol. Anim. Biochim. Biophys.* **18** 893-898
- Kagawa H, Takano K and Nagahama Y 1981 Correlation of plasma estradiol- 17β and progesterone levels with ultrastructure and histochemistry of ovarian follicles in the white-spotted char, *Salvelinus leucomaenis*; *Cell Tiss. Res.* **218** 251-277
- Lambert J G D and van Oordt P G W 1974 Ovarian hormones in teleosts; *Fortschr. Zool.* **22** 340
- Nagahama Y, Clarke W C and Hoar W S 1976 Ultrastructure of putative steroid producing cells in the gonads of coho (*Oncorhynchus kisutch*) and pink salmon (*Oncorhynchus gorbuscha*; *Can. J. Zool.* **54** 1128-1139

- Nagahama Y, Chan K and Hoar W S 1978 Ultrastructure of putative steroid producing cells in the gonads of coho (*Oncorhynchus kisutch*) and pink salmon (*Oncorhynchus gorbuscha*); *Can. J. Zool.* **56** 2508-2519
- Nicholls T J and Maple G 1972 Ultrastructural observations on possible sites of steroid biosynthesis in the ovarian follicular epithelium of two species of cichlid fish, *Cichlasoma nigrofasciatum* and *Haplochromis multicolour*; *Z. Zellforsch* **128** 317-335
- Pearse A G E 1968 *Histochemistry* (London : Churchill Ltd.)
- Polder J J W 1964 On the occurrence and significance of atretic follicles (preovulatory corpora lutea) in ovaries of Bitterling *Rhodeus amarus* (Bloch); *Prod. Kon. Ned. Akad. Net.* **67** 218-222

Development of the incretory organs in the eyestalk of freshwater prawn, *Macrobrachium kistnensis*

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Abstract. The development of the neurosecretory cells in eyestalk is studied in zoea, juvenile and adult stages of the *Macrobrachium kistnensis*. In zoea, the ganglionic mass of the eyestalk comprises of three parts viz. medulla externa, medulla interna and medulla terminalis. The future sensory pore x-organ is characterized by onion bodies near medulla terminalis and a vacuole closely applied to the outer surface of the eyestalk. The monopolar giant neuron is observed in zoea. In juvenile prawn, in addition to zoeal features the sinus gland makes its appearance. At this stage, neurosecretory cells are discernible in the medulla externa and terminalis. In adults, the eyestalk presents well developed lamina ganglionaris, medulla externa ganglionic x-organ (m.e.g.x.), medulla interna ganglionic x-organ (m.i.g.x.) and medulla terminalis ganglionic x-organ (m.t.g.x.).

Keywords. *Macrobrachium*; incretory organs; lamina ganglionaris; medulla externa ganglionic x-organ (m.e.g.x.); medulla terminalis ganglionic x-organ (m.t.g.x.); giant neuron.

1. Introduction

The neurosecretory system of the eyestalk plays an important role in hormonal regulation in crustaceans (Adiyodi and Adiyodi 1970). However, only a few works deal with the development of these neurosecretory centres. Pyle (1943) was the first to describe the histogenesis and cyclic phenomena of the sinus gland and x-organ in *Homarus americanus* and *Pinnotherus maculatus*. Later, Dahl (1957) in *Crangon allamani* and Matsumoto (1958) in *Potamon dehani* studied the embryology of the eyestalk. Hubschman (1963) and Elofsson (1969) gave a detailed account of the development of neurosecretory sites in the eyestalk of *Palaemonetes* and *Penaeus duorarum*, respectively. Recently, Bellon-Humbert *et al* (1978) reported the development and location of neurosecretory and sensory sites in larva and post-larva of *Palaemon serratus*. Most of the studies, reported so far, are mainly concerned with marine crustaceans. Freshwater species have received only a meagre attention in this regard. Therefore, the present study was undertaken to trace the development of the incretory organs in the freshwater prawn, *Macrobrachium kistnensis* from zoea to adult.

2. Material and methods

Macrobrachium kistnensis were collected from Kham river, near Aurangabad. The berried females from the collection were maintained separately in glass bowl. After 29–30 days of incubation, the zoea hatched out. The zoea entered the juvenile phase after 10–11 days (Kulkarni 1972). The eyestalks of zoea, juveniles and adults were removed and fixed in the Bouin's fixative for 24 hrs. Paraffin sections of 8 μ m thickness were prepared and stained with Gomori's Aldehyde fuchsin (Ewen 1962) and Mallory's triple stain.

3. Results

3.1. Ontogenesis of the neurosecretory cells of the eyestalk

3.1a. *Zoea* : After hatching (within 24 hrs) the zoea measured 4–5 mm. The eyes were sessile and cuticle of the cephalothorax was continuous over it. Ommatidia were quadrangular with black pigment. Internally the three medullae viz. externa, interna and terminalis were clearly visible (figure 3a). The monopolar neuron was present near the medulla terminalis (figure 3b). The future sensory pore x-organ (in the form of vacuole) was observed near dorsal surface of the eyestalk (figure 3c). It did not show any connection with the onion bodies of the organ of Bellonci which is situated in the ganglionic mass of the medulla terminalis.

3.1b. *Juvenile* : The juveniles ranged between 11 to 14 mm length. In this stage, the incretory structures were more clearly visible in the lamina ganglionaris and three medullae. The sinus gland, which measured 8.5 μ diameter, was present

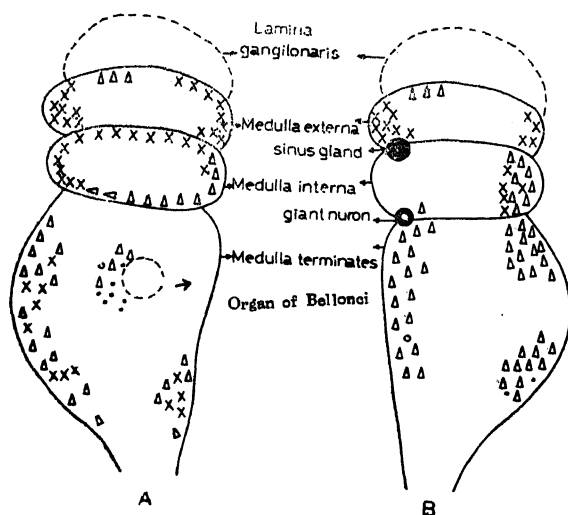


Figure 1. Diagrammatic representation of the eyestalk structures, A—dorsal view and B—ventral view.

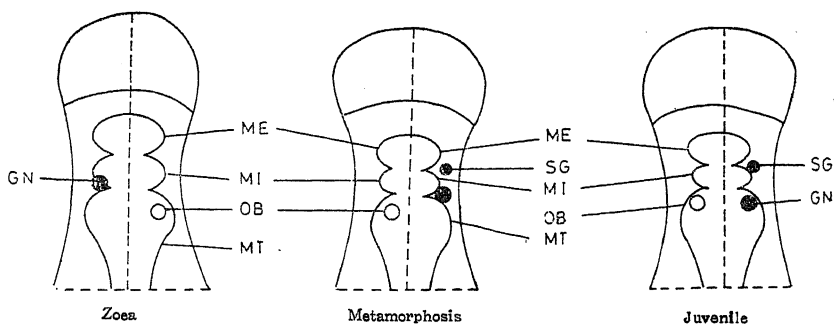


Figure 2. Diagrammatic orientation of the incretory organs of the eyestalk of *M. kistenensis*.

Abbreviations: ME—Medulla externa; MI—Medulla interna; MT—Medulla terminalis; SG—Sinus gland; GN—Giant neuron.

in between medulla externa and medulla interna. It showed distinct affinity for aldehyde fuchsin (figure 4a). The giant neuron measured 12μ in diameter and had 2 to 3 nucleoli (figure 4b). The presumptive neurosecretory sites revealed the neurosecretory cells particularly in the medulla terminalis and medulla interna region (figure 4c, d).

3.1c. Adult eyestalk structure (figures 1, 5a): The optic ganglion consists of four parts: (i) lamina ganglionaris, (ii) medulla externa, (iii) medulla interna and (iv) medulla terminalis. The neurosecretory cells are absent in lamina ganglionaris, but are present in the remaining three parts and are termed as medulla externa ganglionic x-organ (m.e.g.x.), medulla interna ganglionic x-organ (m.i.g.x.) and medulla terminalis ganglionic x-organ (m.t.g.x.). The giant neuron is present in between medulla interna and terminalis (figure 5b). The sinus gland ($21\text{--}26\mu$) is located in between medulla interna and externa. The organ of Bellonci or sensory pore x-organ (spx) is situated in the medulla terminalis region and is embedded in the neurosecretory cells along with onion bodies (figure 5c, d). The organ of Bellonci opens to the outside through the sensory pore (figure 5e).

3.1d. Orientation of the eyestalk incretory structures: During metamorphosis the internal structures of the eyestalk turn through 180° . However, the internal structures retained their original connections to each other. As a result, the dorsal structures are shifted to the ventral side and the ventral structures to the dorsal side in the juvenile prawn. The giant neuron which is present at the dorsal side in zoea turned to the ventral side in the juvenile and the onion bodies also change position, opposite to the giant neuron. The sinus gland in juveniles is located above the giant neuron at medulla externa region. These hypothetical movements of incretory structures of eyestalks are schematically represented in figure 2.

4. Discussion

Hanstrom in 1939 for the first time described the x-organ as 'bunch of grapes' in adult *Homarus americanus*. Pyle (1943) studied the histogenesis of x-organ

in *Homarus americanus* and *Pinnotherus maculatus* and interpreted that the x-organ is found in the eggs and it showed completely developed structure in adults. After these basic studies, the Hanstrom's x-organ or sensory pore x-organ (SPX) or organ of Bellonci was well studied by Hubschman (1963) in *Palaemonetes*, Elofsson (1969) in *Penaeus duorarum* and Jacques (1969 a, b) in stomatopods. They described the cavity under the cuticle in larval stages. In the present study of *Macrobrachium kistnensis* zoea, the SPX was found like a vacuole below the exoskeleton of the eye-stalk. The onion bodies, very few in number, were present in the ganglionic mass of the medulla terminalis. However, Bellon-Humbert *et al* (1978) found the organ of Bellonci with typical onion bodies in the zoeal stage of *Palaemon serratus*. They did not find any vacuole below the cuticle, but described the larval sensory pore (LSP) in zoea, which has no correlation with future SPX. During metamorphosis, the main sensory pore (MSP) established connection with organ of Bellonci.

In adult *M. kistnensis* the well developed SPX is located in medulla terminalis region and opens out through sensory pore. The sensory pore and SPX are connected to each other by a lumen. The sinus gland of juvenile *M. kistnensis* showed positive and negative affinity with AF; means upper part of sinus gland stained purple in colour while lower part stained yellow. The sinus gland of *Palaemon paucidens* (Hisano 1974) stained with Azan (blue, red, vermillion, orange and purple), CHP (pink and gray), AF (AF + ve and AF - ve) and Mallory's triple (blue, orange and greyish green). The giant neuron is a constant feature of the eyestalk during development. Its presence from zoea to adult stage suggests that it may have some role in development, especially after metamorphosis. Again, the occurrence of this giant neuron before the appearance of the neurosecretory centres is significant. Hubschman (1963) described in *Palaemonetes* that the giant neuron may be secretory in nature and the secretion may accumulate in SPX.

The neurosecretory cells in the eyestalk of *M. kistnensis* are distributed in five groups in adult prawns, but two groups (medulla externa and medulla terminalis) show their existence in juveniles. In *Potamon dehani* the neurosecretory cells were well developed before hatching (Matsumoto 1958). In *Astacus leptodactylus* cell types can be distinguished by their colouration at the time of hatching of the eggs (Zielhorst and Van Harp 1976). The present results on *M. kistnensis* agree with the earlier reports of Bellon-Humbert *et al* (1978). They have described the differentiation of the neurosecretory cells of m.e.g.x. and m.t.g.x. with metamorphosis.

The position of sinus gland, giant neuron and SPX in larvae change owing to twisting of medullae along the longitudinal axis of the eyestalk (Hubschman 1963; Elofsson 1969; Elofsson and Dahl 1970; Bellon-Humbert *et al* 1978). In *M. kistnensis* the eyestalk incretory structures, after orientation, retain their constant relations to each other though they are topographically changed. Thus, the ventral side of the larva becomes dorsal in the adult and *vice versa*. The giant neuron passes from distal to proximal position. Above the giant neuron the sinus gland is situated and SPX shifts opposite to it.

Acknowledgement

One of the authors (MSM) expresses her thanks to ICAR, New Delhi, for financial assistance during the completion of research work.

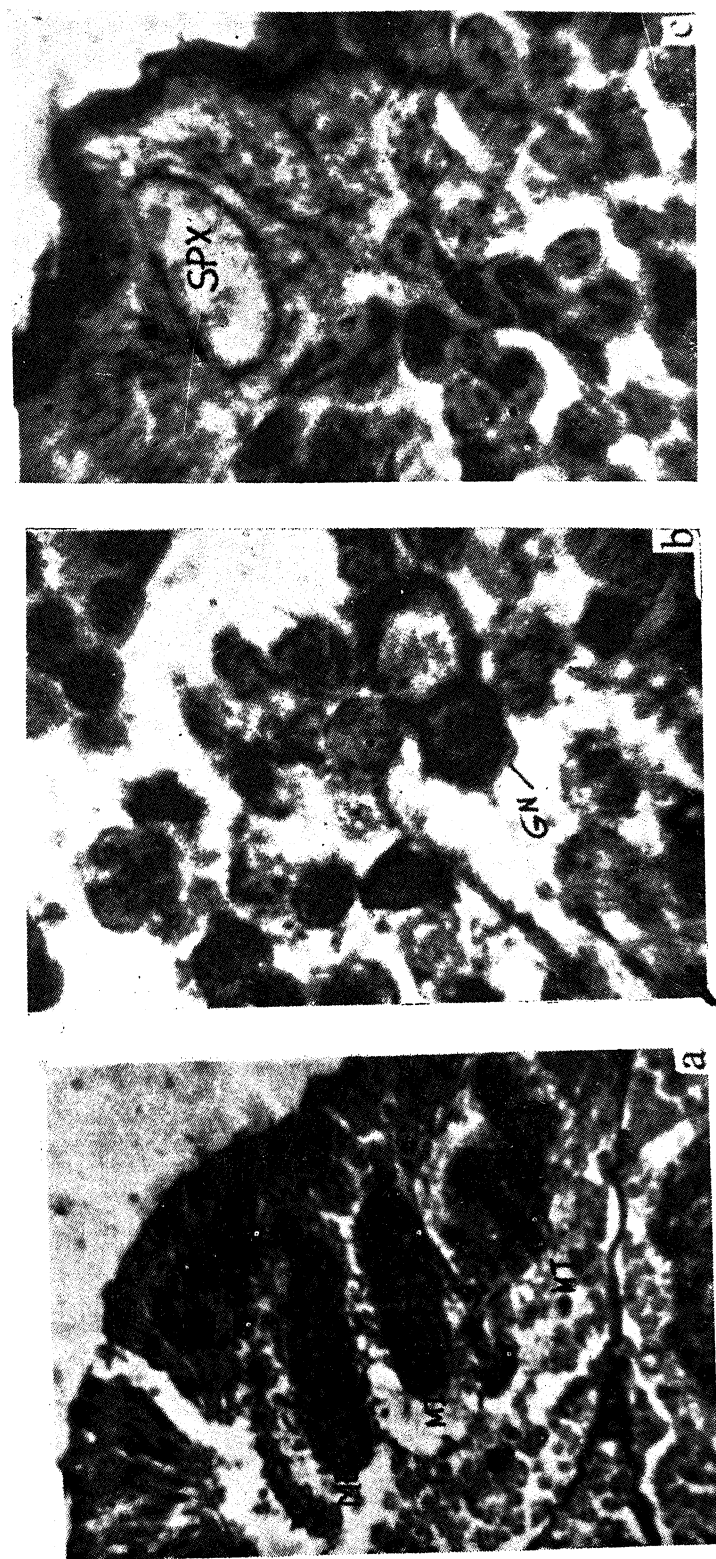


Figure 3. The ontogenesis of the neurosecretory cells in the optic ganglion of the prawn, *Macrobrachium kistnensis*. a. The longitudinal section of the optic ganglion of zoea showing internally, the medulla interna, the medulla externa, medulla terminalis, $\times 60$. b. L.S. of optic ganglion showing the monopolar neuron of the zoea, $\times 400$. c. The sensory pore x-organ (like vacuole) of the zoea, $\times 400$ spx. Abbreviations: MF—Medulla externa, MI—Medulla interna; MT—Medulla terminalis; MN—Monopolar neuron; SPX—Sensory pore x-organ.

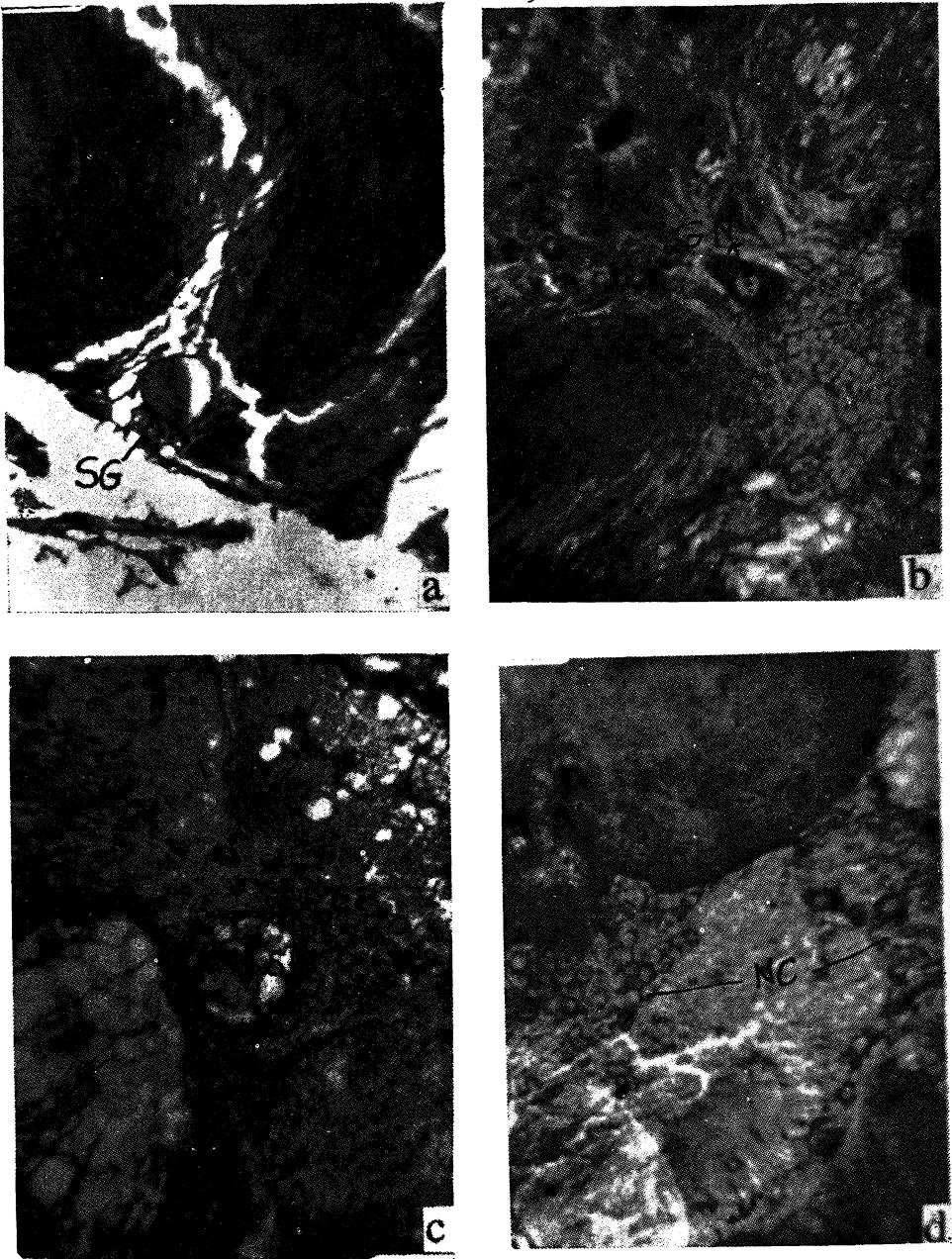


Figure 4. The ontogenesis of neurosecretory cells in the optic ganglion of prawn, *Macrobrachium kistnensis*. a. The sinus gland of juvenile prawn stained with Aldehyde fuchsin, showing AF + ve and 'AF -ve area, $\times 400$. b. The monopolar neuron in the juvenile prawn, stained with Aldehyde fuchsin' $\times 400$. c. The sensory pore x-organ of juvenile prawn, surrounded by neurosecretory cells, $\times 400$. d. The differentiated neurosecretory cells of medulla externa region in juvenile prawn, $\times 400$.

Abbreviations : SG—Sinus gland; GN—Giant neuron; SPX—Sensory pore x-organ; NC—Neurosecretory cells.

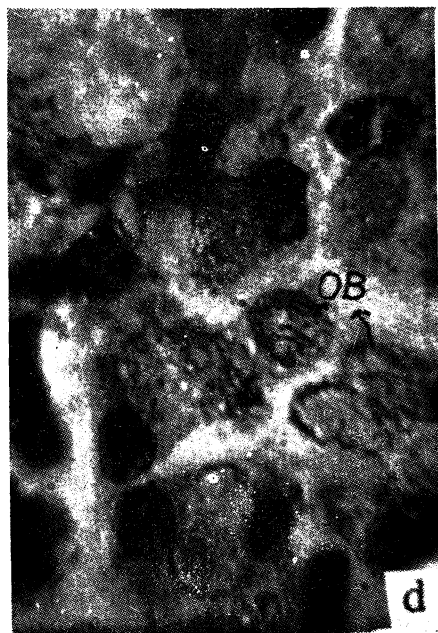
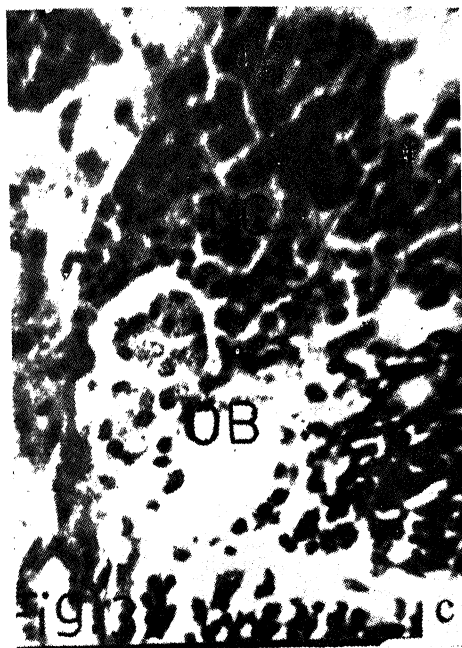
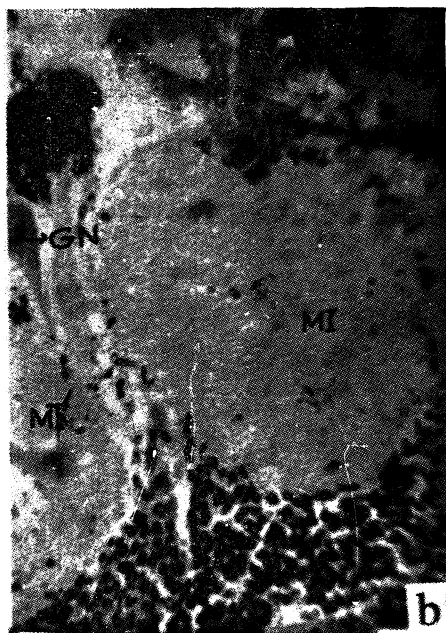
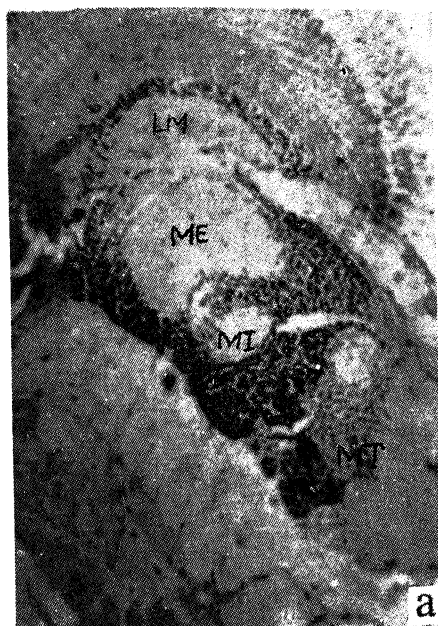


Figure 5.

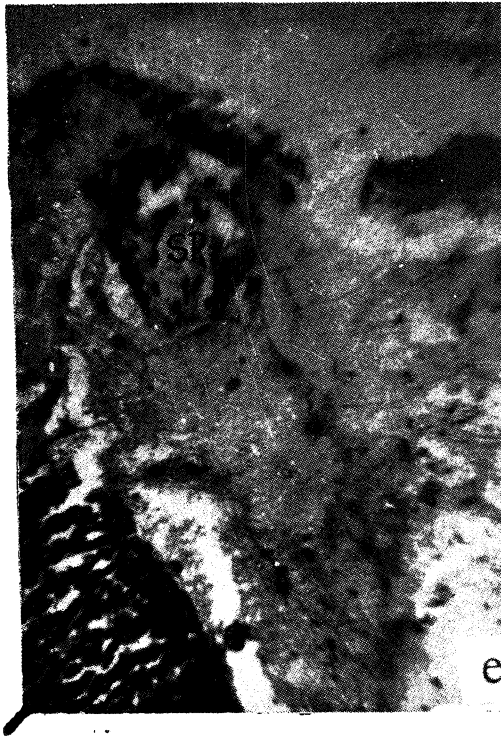


Figure 5e

Figure 5. The eyestalk neurosecretory cells of *M. kistnensis*. **a.** The eyestalk showing lamina ganglionaris, medulla externa, medulla interna and medulla terminalis with neurosecretory cells, $\times 150$. **b.** The giant neuron of the eyestalk situated in between medulla interna and medulla terminalis. **c.** The sensory pore x-organ with onion bodies $\times 150$. **d.** The onion bodies from the sensory pore x-organ, $\times 1000$. **e.** The sensory pore of the sensory pore x-organ, $\times 150$.

Abbreviations: LM—Lamina ganglionaris; ME—medulla externa; MI—Medulla interna; MT—Medulla terminalis. GN—Giant neuron; OB—Onion bodies; NC—Neurosecretory cells; SP—Sensory pore.

References

- Adiyodi R G and Adiyodi K G 1970 Endocrine control of reproduction in decapod crustacea ; *Biol. Rev.* **45** 121-165
- Bellon-Humbert C H, Thijssen N J and Van Harp F 1978 Development location and relocation of sensory and neurosecretory sites in the eyestalks during the larval and post larval life of *Palaemon serratus*; (Pennat) ; *J. Mar. Biol. Assoc. U.K.* **58** 851-868
- Dahl E 1957 Embryology of X-organ in *Crangon allamani* ; *Nature London* **179** 482
- Elofsson R 1969 The development of the compound eye of *Penaeus duorarum* (Crustacea, Decapoda) with remarks on the nervous system ; *Z. Zellforsch. Microsc. K. Anant.* **97** 323-350
- Elofsson R and Dahl E 1970 The optic neuropiles and chiasmata of Crustacea ; *Z. Zellforsch. Microsc. K. Anant.* **107** 343-360
- Ewen A B 1962 An improved aldehyde fuchsin staining techniques for neurosecretory product in insects ; *Trans. Amer. Micro. Soc.* **81** 94-96
- Hanstrom B 1939 *Hormones in invertebrates*, (Clarendon Press, Oxford) i-xii, 1-198
- Hisano S 1974 The eyestalk neurosecretory cell type in the freshwater prawn, *Palaemon paucidens* I A lights microscopic study ; *J. Sci. Hokkaido Univ. Ser. VI. Zool.* **193** 503-514
- Hubschman J H 1963 Development and function of neurosecretory sites in the eyestalk of larval *Palaemonetes* (Decapoda ; Natantia) ; *Biol. Bull.* **125** 96-113
- Jacques F 1969a Existence dun organe sensories dans le pendoncule oculaire des larves de stomatopodes crustace's ; *C.R. Acad. Sci. Paris (Ser. 17).* **268** 89-90
- Jacques F 1969b Histogeneses des pendoncles oculaires des larves de stomatopodes ; *C.R. Acad. Sci. Paris* **20** 565-596
- Kulkarni M Y 1972 Studies on the biology of the freshwater prawn, *Macrobrachium kistnensis* ; Ph. D. thesis, Marathwada University, Aurangabad, India
- Matsumoto K L 1958 Morphological studies on the neurosecretion in crabs ; *Biol. J. Okayama Univ.* **4** 103-176
- Pyle R W 1943 The histogenesis and cyclic phenomena of the sinus gland and X-organ in crustacea ; *Biol. Bull.* **85** 87-102
- Zielhorst A J and Van Harp F 1976 Development dus-systema neurosecreteur du pedoncule oculaire des larves d *Astacus leptodactylus* Nordmann (Crustacea, Decapoda, Reptantia) ; *C.R. Acad. Sci. Paris series D* **283** 1755-1758

Histological observations on tracheal growth during wing development in *Oncopeltus fasciatus* (Dallas) (Heteroptera; Lygaeidae)

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Abstract. The development of the tracheal supply to the larval wing pad of *Oncopeltus fasciatus* is described. The formation of lacunae is also described and it is shown that their development precedes the growth of the associated tracheal supply. Tracheae from anterior and posterior ends of the wing pad enter the lacunae in second instar. The pattern of adult wing tracheation is well established in the third instar. Where the Sub-costa, Radius and Medius arise from Costo-radial trunk, the Cubitus, the first and the second anal tracheae arise from the Cubito-anal trunk. Both groups are connected by a basal transverse connection.

Keywords. *Oncopeltus fasciatus* ; tracheae ; lacunae ; larval instar ; wing development.

1. Introduction

Since the beginning of this century several papers have been published on the histology of insect wing development. While much of the investigation has been confined to Endopterygota (Powell 1904, 1905 ; Marshall 1913 ; Kohler 1932 ; Kuntze 1935 ; Behrends 1935 ; Hundertmark 1935 ; Waddington 1941). Only a small body of literature exists on the wing development of Exopterygotes (Tower 1903 ; Sulc 1911 ; Beck 1920 ; Holdsworth 1940, 1942). Despite the number of works on the histology of wing development, differences of opinion still exist regarding the development of the lacunae, the tracheae and the relations between the two.

In order to investigate the differentiation and the growth of wing epithelia and to ascertain the relationship between the development of lacunae (blood space) and tracheae, a detailed histological study of the large milk-weed bug, *Oncopeltus fasciatus* (Dallas) has been undertaken.

Material and methods

A stock culture of *Oncopeltus fasciatus* at 24–28°C was maintained in plastic containers, on a diet of decorticated sunflower seed and water.

Larval instars of various ages were removed from the cultures and part of thorax and attached wing buds were extirpated and fixed in Bouin's fixative for 18 hrs and dehydrated in ascending ethanol series up to 70%, followed by dioxan (2 changes), and subsequently embedded in paraffin wax. Serial sections 6 μm thick were stained in Mallory's connective tissue stain (Hughesdon's modification) and some in Phosphotungstic haematoxylin (Mallory's). Both of these techniques were found to be good for obtaining full histological details of the developing wings of *O. fasciatus*. The staining times in these solutions were subject to variation, depending on the age of the insect.

The study of earlier stages was made by reconstructions from the serial sections as described by Holdsworth (1942).

3. Results

3.1. Mesothoracic wing

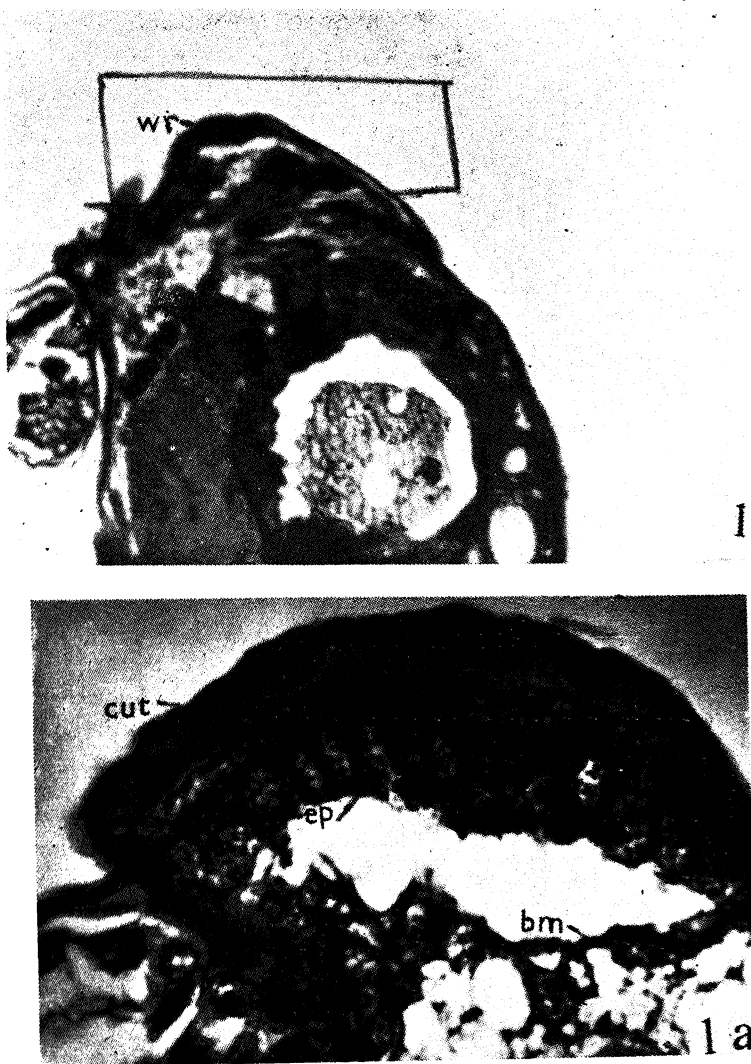
3.1a. *First instar*: Histological study of the thoracic segments of newly hatched first instars (figure 1) reveals the presence of wing pads, that appear like minute flanges, measuring 90–100 μm in length on either side of the thoracic segments. Each rudiment appears as a hollow, flattened outgrowth of the body wall and consists of an outer cuticle, and an inner layer of epidermal cells bounded by a thin, non-cellular basement membrane.

The first instar wing pads are supplied by a single trachea arising from the longitudinal tracheal trunk towards the anterior side of each thoracic segment (figure 9A). It enters the base of the wing pad anteriorly and runs posteriorly terminating between the thorax and the base of the wing disc, measuring 40–50 μm in length and 1–1.5 μm in diameter.

2–4 days after eclosion, the wing pads show a constant gradual growth in their size. As the larva grows, the epidermis detaches itself from the cuticle, the cells elongate, and their ends meet those of the opposite surface (figure 3a, b). A small intercellular space appears in the anterior region of the wing pad, just in front of the trachea, that enters the base of the wing pad and runs across (figure 3b). This trachea is probably the future Costo-radial trunk.

Prior to the first ecdysis, the new cuticle is thrown into folds. The epidermal cells become unilaminar in arrangement again, but the fully formed pharate second instar wing pad is still enclosed in the old cuticle. Though there is single trachea throughout the first instar, in the late pharate second instar, another branch (35–40 μm long and 1–1.5 μm in diameter) has been observed to grow out from the longitudinal tracheal trunk near the posterior margin of the mesothoracic segment (figure 9B) and enters the wing rudiment posteriorly. The two tracheal branches, now lying in the wing pad, grow towards each other.

3.1b. *Second instar*: In the newly moulted second instar, the wing pads on each thoracic segment double in size, 120–130 μm long. In the fully grown



Figures 1. 1a. 1, Section through the mesothoracic wing pad of the newly formed instar $\times 450$. 1a. Wing pad at higher magnification $\times 1800$.

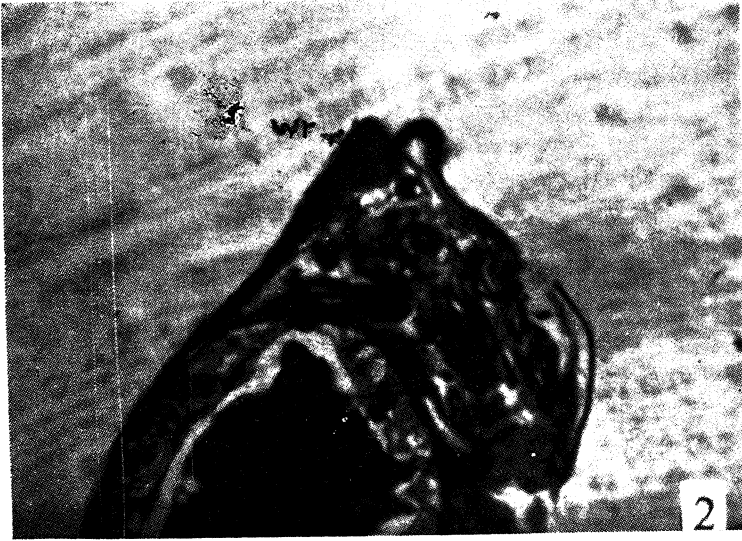


Figure 2. Section through the mesothoracic wing pad of II instar $\times 450$.

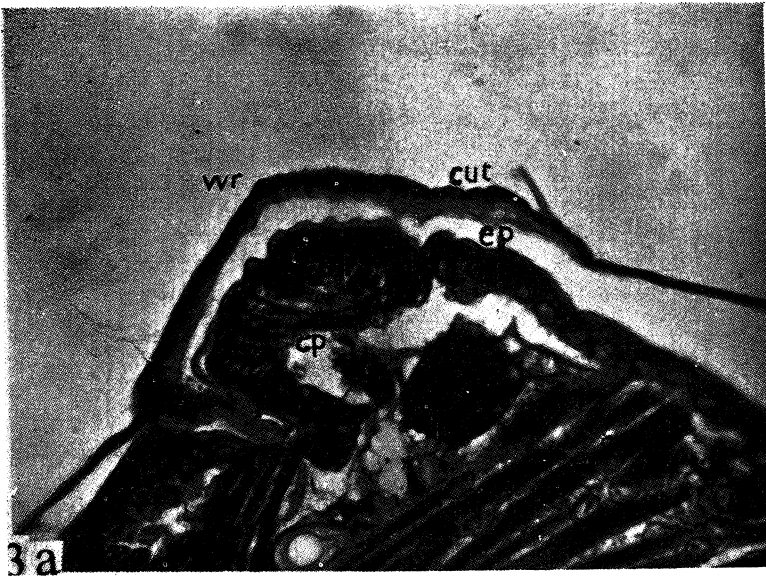
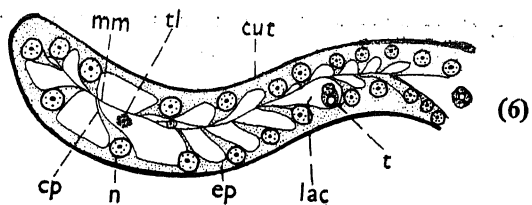
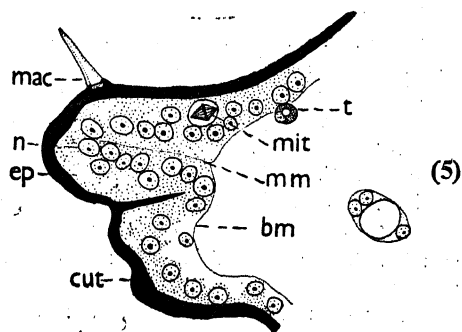
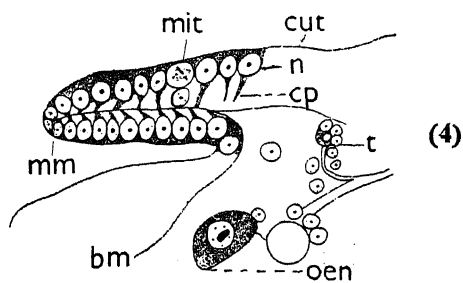
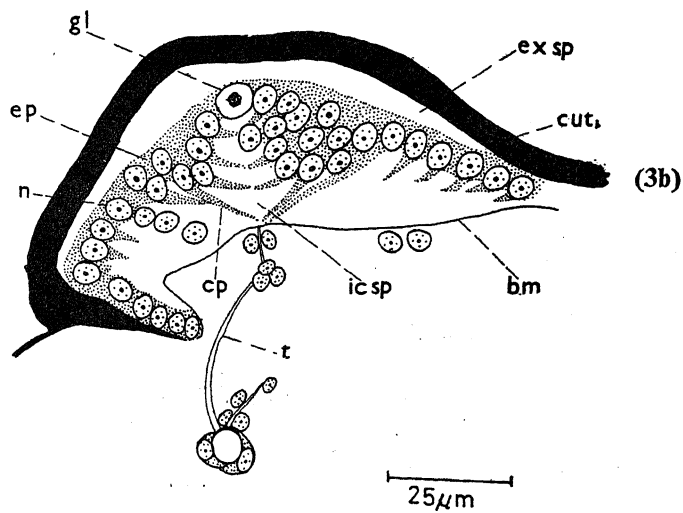
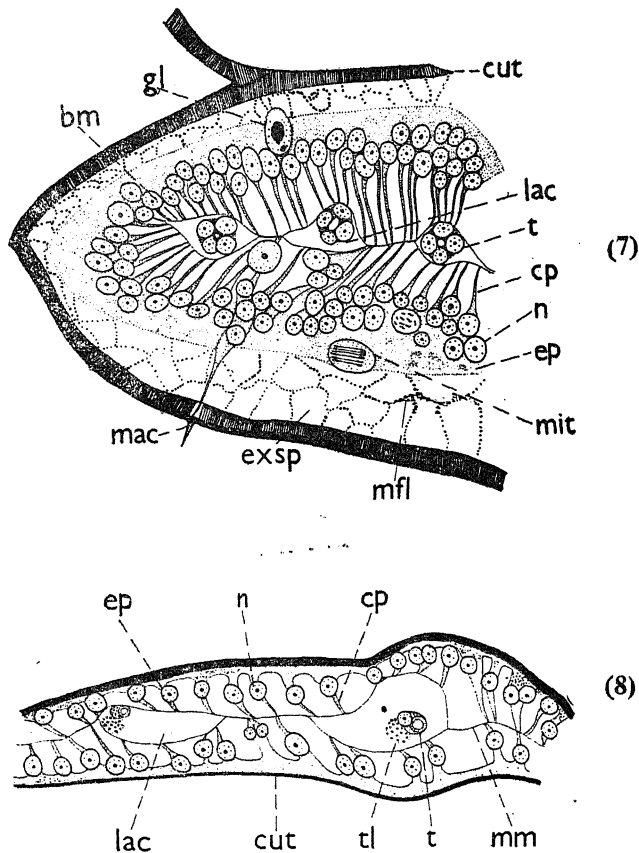


Figure 3. (see captions in p. 614)





Figures 3-8. 3a. Section through the developing wing pad of I instar showing the detachment of the cuticle, elongation of epidermal cells, meeting those of opposite surface $\times 900$. Camera Lucida drawings of : 3b. Section through the developing wing pad of I instar, showing the detachment of cuticle. 4. Section through the II instar wing pad showing the formation of middle membrane. 5. Section through the II instar wing pad after 40 hrs showing mitotic division in epidermal cells. 6. Section through the newly moulted III instar wing pad showing the presence of lacunae. 7. Section through the III instar at 80 hrs after moulting showing five lacunae with their individual tracheae. 8. Section through the IV instar wing pad showing prominent lacunae enclosing tracheae.

Abbreviations : bm: basement membrane; cp : cytoplasmic process ; cut: cuticle; ep: epidermis ; exsp : exuvial space ; gl : gland cell; icsp: intercellular space; lac: lacuna; mac: macrotrichea ; mfl : moulting fluid ; mit: mitotic division ; mm : middle membrane ; n : nucleus ; o : oenocyte ; t : trachea ; tl : tracheole ; wr: wing pad.

second instars, these wing rudiments look like swollen dark areas on the dorso-lateral regions of the thoracic segments (figure 2). The epidermis consists of a single layer of cells ostensibly syncytial in nature. The basement membranes of apposed layers have come close together and a middle membrane has formed but lacunae have not yet developed near the membrane nor are tracheae associated with it. A small space is observed interiorly at the base of the wing pad, just in front of the middle membrane, indicating the beginning of the first formed

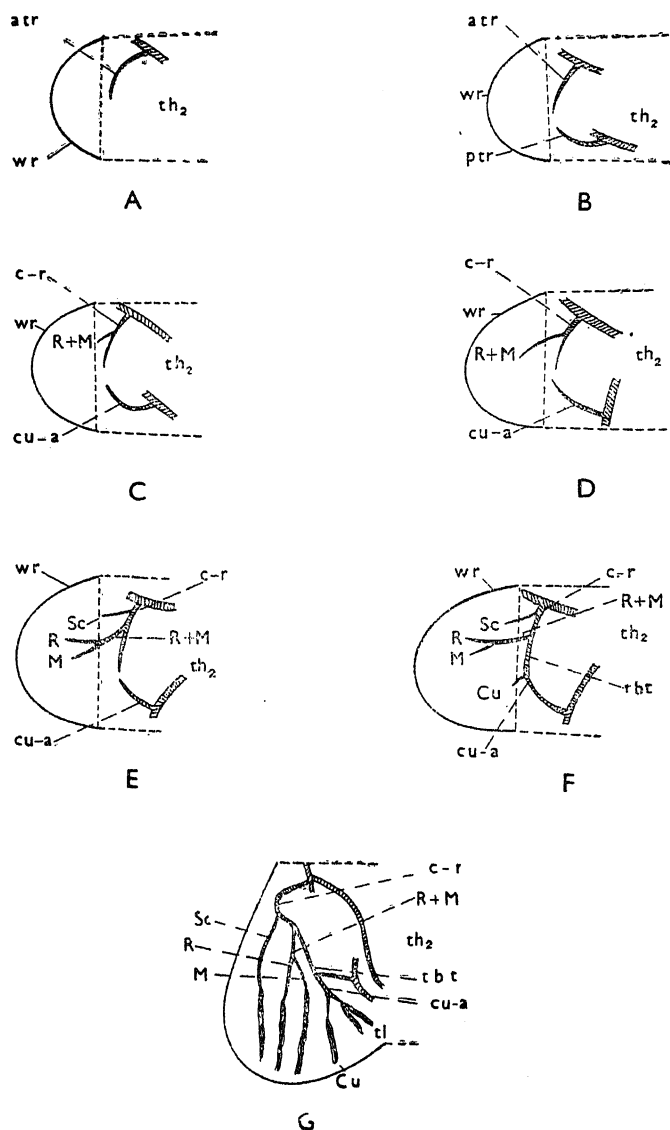


Figure 9. Schematic diagram to illustrate course of tracheae in developing mesothoracic wings from first to third larval instars.

Abbreviations; A. First instar ; B. Late pharate second instar ; C. Newly moulted second instar ; D. 40 hour ; second instar. E. 72 hour second instar ; F. Pharate third instar ; G. Newly moulted third instar : atr: anterior trachea ; c-r : Costo-radial ; Cu : Cubital ; Cu-a : Cubito-anal ; M : Medial ; ptr : posterior trachea ; R : Radial. R + M : radio-medial ; Sc : Sub-costa ; tbt : transverse basal ; th₂ : mesothorax ; tl : tracheoles , wr : wing rudiment.

lacuna (figure 4, *ics_η*). The two tracheae which appeared at the end of the first instar enter from opposite directions of the wing pad, and run across the base. The anterior trachea, a large branch, now measuring $60\mu\text{m}$ in length and $3\text{--}4\mu\text{m}$ in diameter is the Costo-radial trunk. The posterior branch, regarded as the Cubito-anal trunk, now measures $40\mu\text{m}$ in length and $1\cdot5\text{--}2\mu\text{m}$ in diameter. They have grown very close together and are almost in contact, but a transverse basal connection has not yet developed between them. From the Costo-radial trunk, a small branch is seen growing towards the wing pad for the first time (figure 9C). It is only $6\text{--}10\mu\text{m}$ in length at this stage.

The wing pads grow in size ($180\mu\text{m}$ long) and 40 hrs after the first ecdysis, the cells increase in number and the unilaminar arrangement is lost (figure 5). The position of the tracheae is the same as that of the early second instar, and the newly grown branch of the Costo-radial trunk shows a little further extension (figure 9D). At a distance of $18\mu\text{m}$ from its origin from the Costo-radial trunk, this branch penetrates the wing pad for a further $12\text{--}18\mu\text{m}$. It is the first branch to enter the wing pad epithelium and grows into the space at the base of the wing pad which is formed earlier. 72 hrs after ecdysis, this branch penetrates the wing pad epithelium to a depth of about $42\mu\text{m}$ and then bifurcates. Each branch is $24\mu\text{m}$ in length (figure 9E). Just in front of this main branch, a further fine branch has grown from the Costo-radial trunk to form the most anterior tracheal branch of the wing pad, the Sub-costal trachea. The anterior bifurcation of the next branch (*i.e.*, the first to enter the wing pad) is the Radius, and the posterior bifurcation is the Media. The Costo-radial and Cubito-anal trunks have now grown very near to each other (figure 9E).

Prior to the second ecdysis, the Sub-costal, Radial and Medial tracheae show increased growth inside the wing pad. By the coalescence of the trunks, the transverse basal trachea has also been established completely and a separate Cubital trachea has started to grow from the Cubito-anal trunk (figure 9F).

The growth of the metathoracic expansions is rather slow during the second instar and the inner surfaces of the two layers of the wing pad have not fused.

3.1.c *Third-instar* : The wing pads of newly moulted third instar larvae show that the wing membranes of both the surfaces have come to lie very close together and a fused middle membrane is formed by the apposed basement membranes (figure 6). The cuticle covering the wing pad is at first very delicate. The epidermal cells are tall, conical, with oval nuclei, usually towards the base of each cell. The pointed apices of some epidermal cells meet at the middle membrane, while those of others are curved to associate with the ends of neighbouring cells, and leave an intercellular space. Some of these intercellular spaces merge to form lacunae, for the haemolymph. In the newly moulted third instar such spaces even extend up to the tip of wing pad (figure 6). Five prominent lacunae were found in the middle of the wing pad, prior to the appearance of trachea in them. The tracheae at this stage are still very short and confined to a small region at the base of the wing pad.

At this stage the framework of adult tracheation has also been established. The Sub-costa enters the wing pad, just beneath the humeral angle. It is about $120\mu\text{m}$ long and bundles of tracheoles arise from it to supply the tip of the wing pad. These tracheoles are not visible in some sections, but in others they are

very clear. The Radius and the Media run inside the second and third lacunae respectively for a distance of 120–130 μm and give rise to bundles of tracheoles to supply the tip of the wing pad. The anteriormost branch of the Cubito-anal trunk the Cubital trachea, is 6–10 μm long and enters the fourth lacuna of the wing pad (figure 9G). It also gives off bundles of tracheoles. Towards the tip of Cubito-anal trunk, two separate bundles of tracheoles grow out and enter the remaining lacuna to indicate the future growth of the first and second anal tracheae.

By 70 hrs after the second ecdysis the lacunae are very prominent. The anterior-most four tracheae, the Sub-costa, Radius, Media and Cubitus have now extended towards the tip of wing pad, only 40–50 μm away from the apex. The fifth trachea (the first anal trachea) runs parallel to the base.

By 80 hrs after the second ecdysis, the epidermis has reached its maximum thickness (20 μm). Proliferative cell division is still in progress, though it is now about to decline in intensity (figure 7). Though they are very much crowded together to a thickness of several layers, each nucleus is associated with a tail-like process that runs towards the basement membrane. All the five lacunae (Sub-costa, Radius, Medius, Cubitus and 1st Anal) contain their individual tracheae which run up to the tip of the wing pad (figure 9G).

3.1d. *Fourth instar*: In the fourth instar, the cuticle covering the wing pad is very thin and delicate. The epidermis of the wing pad is thin, consisting of a single layer of cells. The basement membranes of the apposed layers remain close together as a middle membrane, except around the lacunae (figure 8). The five prominent tracheae running throughout the length of the wing pad grow to about 150 μm from the tip. Now there is also a well established sixth lacuna which runs parallel to the base of the wing pad and is occupied by a second anal trachea. It is fully grown in the late fourth-instar. All six lacunae correspond in arrangement with the veins of the adult wings, which will be formed later by differential sclerotisation of the integument adjacent to the lacuna. By this time, the basic pattern of adult venation and tracheation have been established completely. Groups of tracheoles arise from each nodal point in order to supply air to every part of the wing pad.

Except for their diameter and length, the pattern of tracheation is same in the fifth instar wing pads and in newly moulted adult wings (figures 11, 13).

3.2. *Metathoracic wing*

The essential features of the tracheal growth in a developing metathoracic wing are identical to those of a mesothoracic wing (figure 10). The rudiments of both anterior and posterior trachea arise at the end of 1st instar, which penetrate into the anterior Costo-radial trunk and a posterior Cubito-anal trunk. During the second instar a transverse basal connection is established between them, and a branch each from the Costo-radial and Cubito-anal tracheae penetrate the wing pad epithelium (figures 10D, E). A lacuna develops prior to the second ecdysis. During the third instar stage, the wing pad development records a rapid growth. The tracheal branching is fully established. Six lacunae develop and the tracheal branching penetrates these lacunae. In the fourth instar stage,

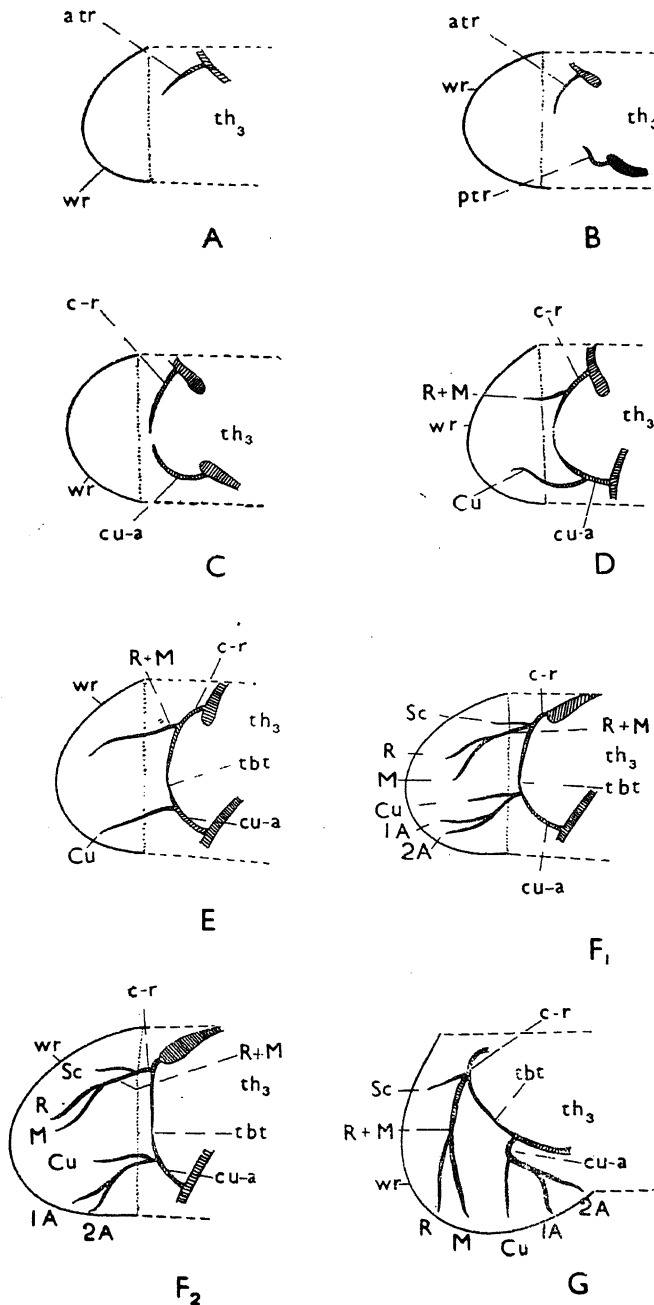


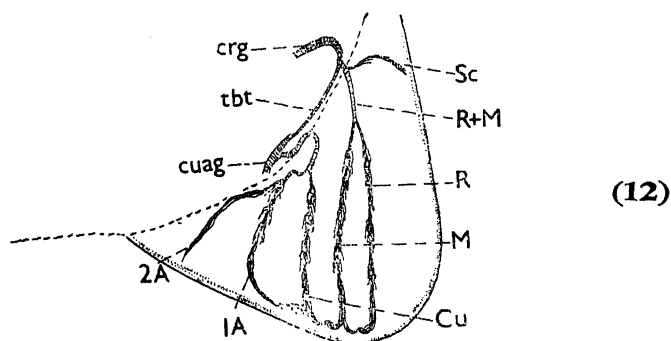
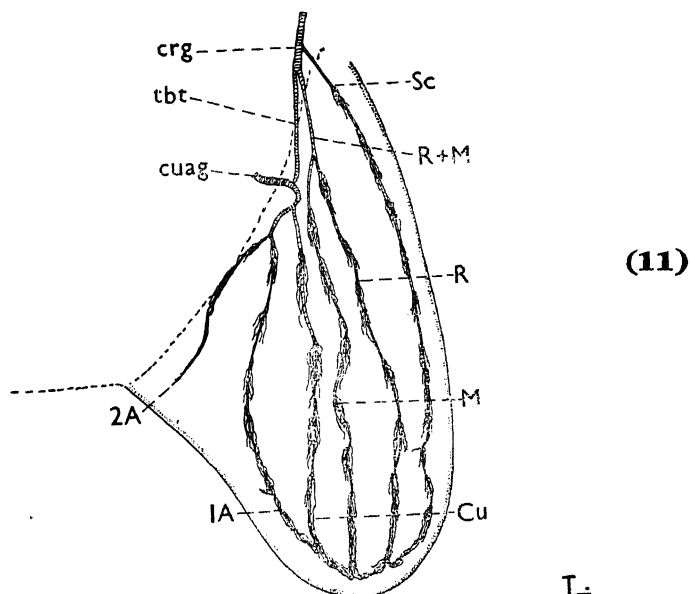
Figure 10. Schematic diagram to illustrate course of tracheae in developing meta-thoracic wings from first to fourth larval instars.

Abbreviations: A : first instar ; B : Late pharate second instar. C : Newly moulted second instar ; D : Late pharate third instar ; E : Newly moulted third instar ; F₁F₂ : Pharate fourth instar ; G : Newly moulted fourth instar. ; 1A, 2A : anal tracheae ; atr : anterior trachea ; C-r : Costo-radial ; Cu : cubital ; Cu A : Cubito-anal ; M : Medial ; ptr : posterior tracheae ; R : Radial ; R + M : Radio medial ; Sc : Subcostal ; tbt : transverse basal ; th₃ : metathorax ; wr : wing rudiment,

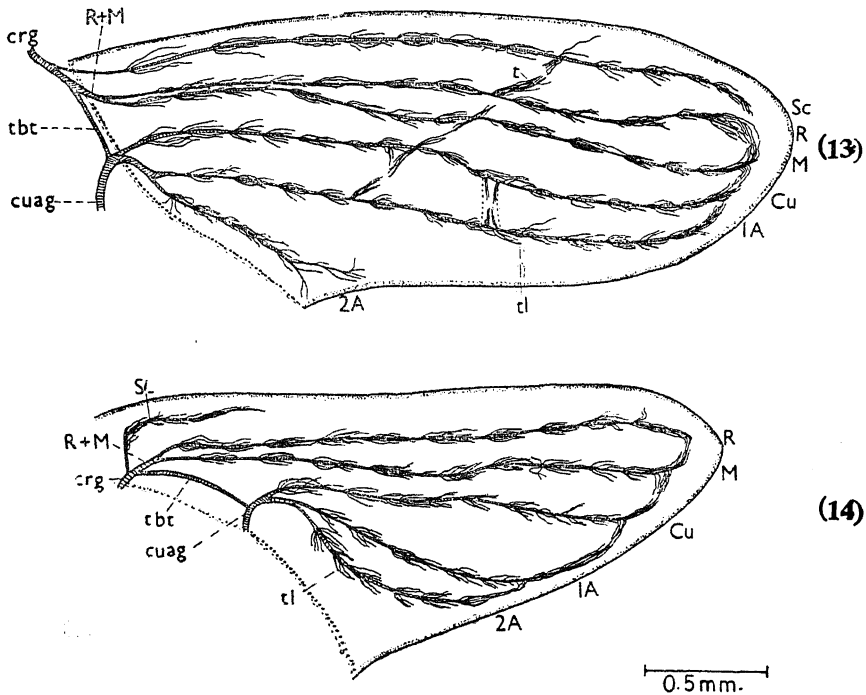
the two tracheal trunks are joined by the transverse basal connection. The tracheal ramifications are complete and the entire tracheal system assumes the framework of an adult wing. In the fifth instar, the basic pattern is the same as that of the fourth instar, except an increase in the length of trachea and in the number of tracheoles (figures 12, 14).

4. Discussion

It is established that the lacunar system corresponds to the venational system. But opinions differ regarding the developmental relationship of the lacuna and the trachea. Marshall (1913) while studying *Platyphylax designatus* (Tricheoptera)



Figures 11-12. 11. Tracheation of right fore wing pad of fourth instar larva of *Oncopeltus* (including basal connection). 12. Tracheation of right hind wing pad of fourth-instar larva.



Figures 13-14. 13. Tracheation of fifth instar fore wing pad (including basal connections). 14. Tracheation of hind wing pad of fifth instar larva of *Oncopeltus* including basal connection. The sub-costa is a very small trachea.

Abbreviations: 1A, 2A : anal ; crg : Costo-radial ; Cu : Cubital ; Cuag : cu bito-anal ; R : Medial ; R + M : Radio-medial ; Sc : Sub-costal ; t l : tracheoles ; tbt : transverse basal.

stated that the lacunae develop in the wing pad of the last larval stage and that the tracheae do not enter until the wing is everted at pupation. Hundertmark (1935), while dealing with the histology of *Tenebrio molitor* wings, reported that the tracheae grow into the newly everted wing disc and later the lacunae are formed about them. Kuntze (1935) in his studies of *Philosamia cynthia* (Lep.) observed the formation of the lacuna earlier than the entrance of trachea. All these are Endopterygotes. Holdsworth (1940, 1942) in his histological studies of the wing pads of *Pteronarcys proteus* (Plecoptera) found that the precursors of the veins in the nymphal wing pad are the lacunae, the free spaces surrounded by spongy columnar epidermal cells. The trachea and nerves grow into these channels only after their patterns have been established.

In the early first instar wing pads of *O. fasciatus* the epidermal cells increase in size and undergo mitotic division. The divided cells elongate and their inner ends meet those of the opposite surface. During this stage a small inter-cellular space is seen towards the anterior region of the wing pad. The subsequent formation of lacunae in the wings of *O. fasciatus* is quite similar to that of *Pteronarcys proteus* (Holdsworth 1940, 1942). The tracheae grow into these lacunae indicating that even in *O. fasciatus* lacunar development precedes the entrance of

tracheae. Holdsworth's conclusions are, therefore, extended to another quite unrelated order of Exopterygota. Whether the condition found by Hundertmark (1935) is a secondary specialization found in Coleoptera or whether there exist considerable variations in the sequence of lacunar and tracheal development, require more elaborate study.

The growth of metathoracic wing pads is slow during the second instar, but in both meso and metathoracic wing pads complete pattern of adult tracheation is well established in the fourth instar.

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References

- Beck H 1920 Die Entwicklung Flugelgeaders bei *Phyllodromia* (Blatta) Germanica; *Zool. Jahrb. Anat.* 41 377-410
- Behrends J 1935 Ueber die Entwicklung des Lakunen. Ader- und Tracheen systems Während Puppenruhe im Flügel der Mehlmotte *Ephesia kühniella* Zeller; *Z. Morph. Okol. Tiere.* 30 573-596
- Holdsworth R P 1940 The histology of the wing-pads of the early instars of *Pteronarcys proteus* Newport (Plecoptera); *Psyche.* 47 112-119, 714-715
- Holdsworth R P 1942 The wing development of *Pteronarcys proteus* (Pteronarcidae: Plecoptera); *J. Morphol.* 76 431-462
- Hundertmark A 1935 Die Entwicklung der Flügel des Mehlkäfers *Tenebrio molitor*, mit besonderer Berücksichtigung der Hautungsvorgänge; *Z. Morphol. Okol. Tiere.* 30 506-543
- Köhler W 1932 Die Entwicklung der Flügel beider Mehlmotte *Ephesia kühniella* Zeller mit besonderer Berücksichtigung des Zeichnungsmusters; *Z. Morphol. Okol. Tiere.* 24 282-681
- Kuntze H 1935 Die Flügelentwicklung bei *Philosamia cynthia* Drury, mit besonderer Berücksichtigung des Geaders der Lakunen und der Tracheensysteme; *Z. Morphol. Okol. Tiere* 30 544-572
- Mallela N 1979 Histology and ultrastructure of wing development in *Oncopeltus fasciatus* (Dallas) (Hemiptera: Heteroptera); unpublished thesis, London University, London.
- Marshall W S 1913 The development of a caddis fly, *Platyphylax designatus* Walker; *Z. f. Wiss. Zool.* 105 574-597
- Powell P B 1904, 1905 The development of wings of certain beetles and some studies on origin of wings of insects; *J. New York Entomol. Soc.* 12, 13 237-243, 5-23
- Sulc K 1911 Über Respiration, Tracheen system, Und Schaumproduktion der Schaumzikaden Larven; *Z. f. Wiss. Zool.* 99 147-188
- Tower W L 1903 The origin and development of the wings of Coleoptera; *Zool. Jahrb. Anat.* 17 517-572
- Waddington C K 1941 The genetic Control of wing development in *Drosophila*, *J. Genet.* 41 71-139

The functional demography of adrenal glands in *Rattus meltdada pallidior* in Indian desert

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Abstract. In this paper, the seasonal fluctuations in adrenal glands and its relationship with their body weights, reproduction activity and population density of soft-furred field rat, *Rattus meltdada pallidior* in the Indian desert has been discussed. Results of the present study revealed that the left adrenal gland in both the sexes of rodents was found to be significantly heavier ($P < 0.01$) than the right one. The paired adrenal of female rats was significantly heavier ($P < 0.001$) than those of males. The seasonal variations in adrenal weights of pregnant females were found to be significantly heavier ($P < 0.01$) than those of nonparous females. The adrenal weights of male, pregnant and non-pregnant female rats were found to be significantly correlated with their body weights. Results of this study further revealed that changes in adrenal weights in *Rattus m. pallidior* are functions of body weights which are regulated by the availability of food and its nutritional level.

Keywords. *Rattus meltdada pallidior* ; adrenal glands ; body weight ; pregnant females ; reproduction activity ; population density.

1. Introduction

It has been postulated that adrenal weight is the function of population density in Albino rats and in wild house mice of confined density (Christian 1955 ; 1956 ; Christian and Davis 1955). Clarke (1953) , Christian (1962) and Southwick (1963) stated that fighting and social-interactions enhance adrenal weights. Contrarily, Southwick (1958) and Rudd and Muthen (1963) did not observe adrenal enlargements due to fighting in house mice (*Mus musculus*) and Pocket gophers (*Thomomys umbrinus*) respectively.

In view of the confirmity, the present study on relationship of adrenal gland with their body weights, reproduction activity and population density was undertaken in the free living population of soft-furred field rat, *Rattus meltdada pallidior* in Thar desert.

2. Materials and methods

The *Rattus meltada pallidior* (45 ♂♂ and 43 ♀♀) were captured during January 1978 to December 1978 from Bisalpur (25° 7' N-73° 10' E) in Western Rajasthan. Later on, the rats were weighed, sexed, dissected and both right and left adrenal glands were preserved in 10% formaldehyde. The preserved adrenal glands were weighed on semimicro balance to the nearest 0.001 g.

3. Results

3.1. Difference between right and left adrenal gland

The left adrenal gland was found to be significantly heavier than the right one in both the sexes of rats (table 1).

3.2. Difference between sexes

The right and left adrenal gland of female rats were found to be heavier than those of the males, but the significant ($P < 0.001$) difference was noticed in case of left ones (table 1). Similarly, on an average the paired adrenal weights of female rats were significantly ($P < 0.01$) heavier than those of male rodents. The mean monthly paired adrenal weights of females were found to be significantly heavier ($P < 0.05$, $P < 0.01$) than those of male rats, almost throughout the year. However, the male adrenals were recorded significantly heavier ($P < 0.01$) during July and October (table 2). The relative as well as absolute adrenal weights of pregnant females were observed significantly heavier ($P < 0.01$) than those of nonparous females (table 3).

3.3. Seasonal trend through the year

The fluctuations in adrenal weights of both male and female exhibit a peak during February, July and October in former sex and February-March and September-

Table 1. Absolute adrenal weights (mean \pm S.E.) of *Rattus meltada pallidior*.

Sex	No.	Average adrenal weights (mg)			
		Right	Left	Paired	't' between
Male	45	6.98 \pm 0.63 (1)	8.52 \pm 0.71 (3)	13.46 \pm 1.05 (5)	1 and 3 = 2.65 ($P < 0.01$)
Female	43	7.79 \pm 0.42 (2)	9.55 \pm 0.47 (4)	16.48 \pm 2.41 (6)	2 and 4 = 3.74 ($P < 0.001$) 3 and 4 = 2.03 ($P < 0.05$) 1 and 2 = 1.44 5 and 6 = 5.74

Table 2. Seasonal fluctuations in adrenal weight of *Rattus meltada pallidior*.

Months	Paired adrenal weights Mean \pm S.E.		't' between males and females
	Males (n = 45)	Females (n = 43)	
January	10.12 \pm 5.95	9.22 \pm 5.30	0.39
February	18.65 \pm 0.46	17.53 \pm 8.04	0.44
March	10.00 \pm 0.00	18.55 \pm 0.75	5.09**
April	9.44 \pm 2.05	13.10 \pm 2.06	4.93**
May	9.00 \pm 0.00	13.00 \pm 1.00	2.85*
June	12.36 \pm 2.58	14.96 \pm 3.58	2.27*
July	23.18 \pm 2.58	14.96 \pm 3.58	2.27*
August	10.90 \pm 1.35	16.55 \pm 2.08	2.09*
September	11.00 \pm 0.00	19.90 \pm 0.07	5.83**
October	18.55 \pm 1.50	16.50 \pm 1.50	2.13*
November	15.83 \pm 0.58	22.25 \pm 2.20	3.02*
December	12.51 \pm 3.51	18.05 \pm 1.88	3.40*

* = $P < 0.05$; ** = $P < 0.01$

November in latter sex (table 2). Thereafter, in females, they remained almost constant throughout the year. Whereas, in case of males remarkable decrease from March to June was observed. The lowest adrenal weights were found during August to September in case of males (table 2).

Table 3. Adrenal weight in relation to prevalence of pregnancy.

Adrenal weight	Adult females		't' between
	Pregnant (n = 28)	Nonparous (n = 15)	
Absolute	18.35 \pm 1.32 (1)	16.52 \pm 0.60 (2)	1 and 2 = 3.21**
Relative	42.14 \pm 0.93 (3)	38.48 \pm 1.38 (4)	3 and 4 = 5.94**

** = $P < 0.01$

3.4. Adrenal weight in relation to body weight

The adrenal weights of male, pregnant and not pregnant female metads were found to be significantly correlated ($r = + 0.592$, $P < 0.01$, $r = + 0.609$, $P < 0.01$ and $r = + 0.890$, $P < 0.01$) respectively with their body weights. The fluctuations in the changes of male and female adrenal weights were found to be almost in similar pattern throughout the year (figures 1 and 2), which suggests that weight of adrenal gland in *Rattus meltda pallidior* is influenced by body weight.

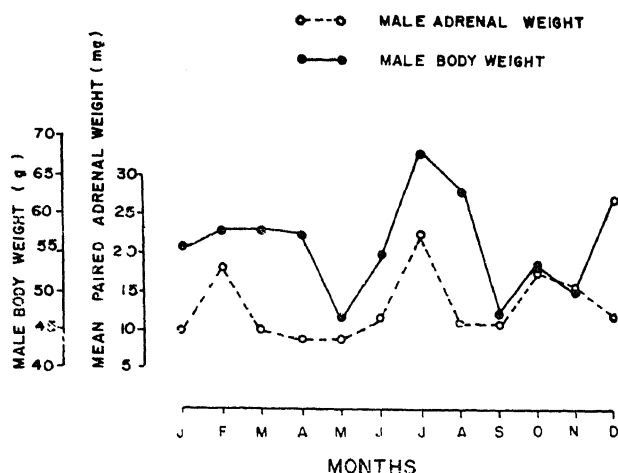


Figure 1. Paired adrenal weights of male *Rattus m. pallidior* in relation to their body weight.

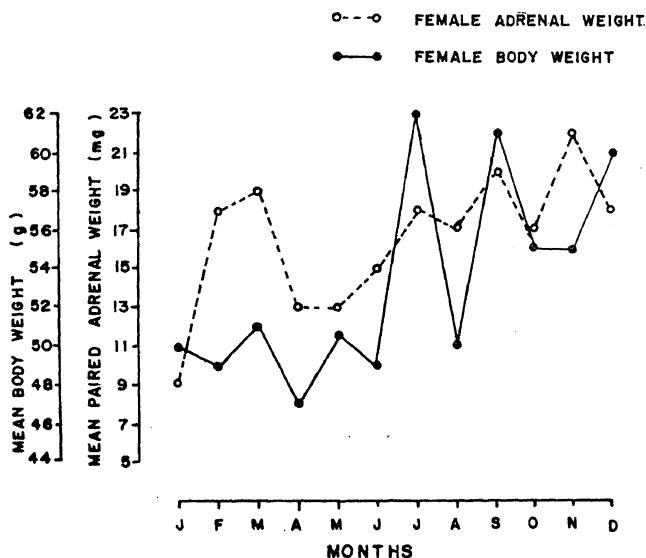


Figure 2. Paired adrenal weights of female *Rattus m. pallidior* in relation to female body weight.

3.5. Adrenal weight in relation to reproduction activity

The females *R. m. pallidior* litter throughout the year with two peaks one in March to April and another in July to November (Rana and Prakash 1981). The adrenal weights of females *R. m. pallidior* show a parallel fluctuation trend with the prevalence of pregnancy (figure 3), suggesting an increase in adrenal weights with the enhanced female fertility. The adrenal weights are influenced by pregnancy stress is further confirmed by the data presented in table 3, where both the absolute as well as relative adrenal weights of pregnant female rodents are significantly heavier ($P < 0.01$) than those of nonparous females (table 3).

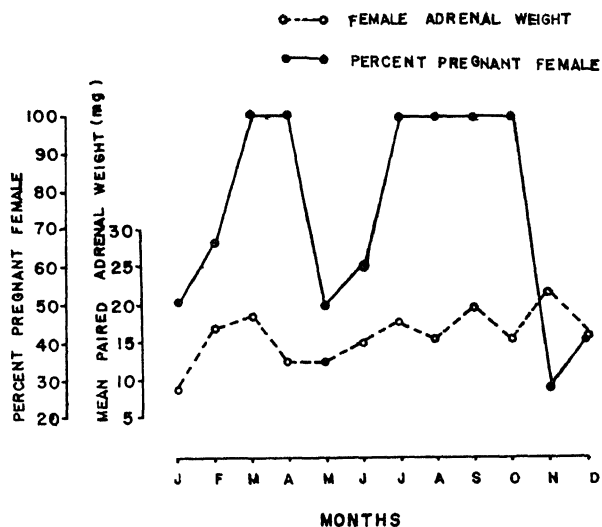


Figure 3. Paired adrenal weights of *Rattus m. pallidior* in relation to female fertility.

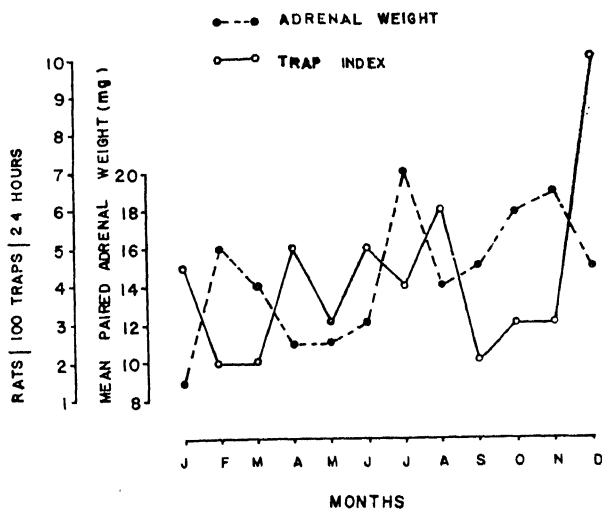


Figure 4. Variation in paired adrenal weights of *Rattus m. pallidior* in relation to their population density.

3.6. Adrenal weight in relation to population density

The pooled adrenal weights of both male and female rats showed three peaks in July, November and February, whereas, the population density was found to be low during these peak levels, indicating reverse pattern (figure 4). This may be explained by the facts that their numbers do not influence the seasonal variations among adrenal gland. The trap indices exhibited two peaks, one in December and the second in April–June and August. These peaks do not have any relationship with their adrenal weights.

4. Discussion

A striking similarity in the fluctuations of adrenal weight and body weight in adult male and female indicate that changes in variations among them are influenced by body weights which are regulated by the availability of food and its nutritional level. Similar observations were made among other species of Indian mammals (Prakash *et al* 1969 ; Jain 1971 ; Rana *et al* 1975 : Rana 1981).

Selye (1936) argued that an increase in adrenal weight is due to the pregnancy stress, on the other hand, Christian (1962), Christian and Davis (1964) suggested that this enhancement is a reflection of the social-interactions, the frequency of which usually exhibited an increase during breeding season. Similarly, in the present study the significant differences in the adrenal weights of pregnant and non-parous *R. meltda* points out that physiological processes of reproduction might have an impact on the adrenal weights. Similar observations were made in *R. c. cutchicus* (Rana *et al* 1975), *T. indica* (Jain 1971) and Jack-Rabbit, *Lepus californicus melanotis* (Herrick 1965). Whereas no relationship was found between male fecundity and adrenal weights decreased (figure 5), however, percent

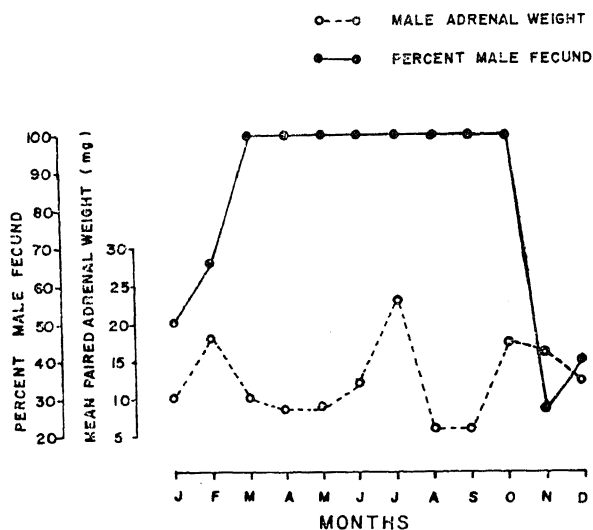


Figure 5. Paired adrenal weights of *Rattus m. pallidior* in relation to male fecundity.

male fecund rats showed a decline trend during November. The adrenal glands did not reflect much change in their seasonal trend. During summer season when breeding activity in male metad had ceased, a second minor peak in adrenal weights was exhibited.

The average number and the adrenal weights of Woodchucks, *Marmota monax*, are closely associated and fluctuations found among them follow a parallel trend throughout the year which tends to indicate that weight of adrenal glands is influenced by population density (Christian 1962). Whereas, the results of present study on free living population of *R. meltada* suggest that the increase in population density decreased the weight of adrenal gland.

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References

- Christian J J 1955 Effect of population size on the adrenal glands and reproductive organs of male mice in population of fixed size ; *Am. J. Physiol.* **182** 292-300
- Christian J J 1956 Adrenal and reproductive responses to population size in mice from freely growing populations ; *Ecology* **37** 258-273
- Christian J J 1962 Seasonal changes in the adrenal glands of Woodchucks (*Marmota monax*) ; *Endocrinol.* **7** 431-447
- Christian J J and Davis D E 1955 The reproduction of adrenal weight in rodents by reducing population size ; *Trans. 20th North Am. Wildlife Conf.* pp.177-184
- Christian J J and Davis D E 1964 Adrenal glands in female voles (*Microtus pennsylvanicus*) as related to reproduction and population size ; *J. Mammal.* **47** 1-18
- Clarke J R 1953 The effect of fighting on the adrenals, thymus, and spleen of the vole (*Microtus agrestis*) ; *J. Endocrinol.* **9** 114-126
- Herrick E H 1965 The black-tailed Jack rabbit Kansas N. Endocrine Studies ; *Kansas Univ. Sci. Bull.* **140** 73-75
- Jain A P 1971 Adrenal weight in the Indian gerbil, *Tatera i. indica* Hardwicke, as related to bodyweight and reproduction activity ; **4** 279-288
- Prakash Ishwar, Dass B and Taneja G C 1969 Adrenal weight in relation to body weight and reproduction activity in the Indian desert hare, *Lepus nigricollis dayanus* ; *J. Anat. Soc. India* **2** 42-47
- Rana B D 1981 Variations in the weight of the adrenal glands of *Suncus murinus sindensis* ; *Annals Zool.* **18** 115-124
- Rana B D and Prakash Ishwar 1981 Reproduction biology of the soft-furred field rat, *Rattus meltada pallidior* (Ryley 1914) in the Rajasthan desert ; *J. Bombay nat-Hist. Soc.* (In press).
- Rana B D, Prakash Ishwar and Jain A P 1975 Variation in the weight of adrenal glands of Cutch-Rock rat, *Rattus cutchicus* (Wroughton) ; *Mammalia* **39** 479-486
- Rudd R L and Muthen D A 1963 Adrenal gland responses to experimental manipulations of captive pocket gophers (*Thomomys umbrinus*) ; *J. Mammal.* **44** 451-466
- Selye H 1936 Thymus and adrenals in response of the organism to injuries and intoxications ; *Br. J. Expt. Pathol.* **17** 234-248
- Southwick C H 1958 Population characteristics of house mice living in English corn ricks : density relationships ; *Proc. Zool. Soc. London* **131** 163-175
- Southwick C H 1963 Adrenal weights and eosinophil levels of *Peromyscus leucopus* in relation to social climate ; *Bull. Ecol. Soc. Am.* **44** 130 (Abstr.)

Description of three new species of *Drosophila* (*Scaptodrosophila*) from Orissa, India

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Abstract. *Drosophila koraputae*, *D. neomedleri* and *D. puriensis* all belonging to the subgenus *Scaptodrosophila* are described as new species. Their taxonomic relationships, based on the morphology and male genital structures, are established.

Keywords. Drosophilidae ; *Drosophila koraputae* ; *D. neomedleri* ; *D. puriensis*.

1. Introductions

Until recently very little has been known concerning the Drosophilid fauna of Orissa (Gupta 1972 ; Dasmohapatra *et al* 1981). This paper deals with the descriptions of three more new species collected recently from a wild area in Koraput district of Orissa.

2. Taxonomic descriptions

2.1. Genus *Drosophila* Fallen

Drosophila Fallen, 1823, Diptera *Sueciae Geomyzides*, 2 : 4 Type species : *Musca funebris* Fabricius ; Sweden.

2.2. Subgenus *Scaptodrosophila* Duda

Scaptodrosophila Duda, 1923, Mus. Nat. Hungarici, Ann. 20 : 37. Type species : *Scaptodrosophila*.

Scaptomyzoidea Duda ; New Guinea

Paradrosophila Duda, 1923, Mus. Nat. Hungarici, Ann. 20 : 43. Type species : *Drosophila pictipennis* Kerte'sz ; New Guinea.

Pugiodrosophila Duda, 1924, Arch. Naturg. 90A (3) : 203. Type species : *Drosophila pugionota* de Meijere ; Simalur.

Xiphidiochaeta Duda, 1925, Mus. Nat. Hungarici, Ann. 22 : 200 (improper replacement name for *Pugiodrosophila* ; type : *D. pugionota* de Meijere).

Pholadoris Sturtevant, 1942, Univ. Texas publ. 4213 : 28. Type species : *Drosophila victoria* Sturtevant ; United States.

2.3. *Drosophila* (*Scaptodrosophila*) *koraputae*, sp. nov.

2.3a. *Head*, ♂ and ♀ : Arista with 4 dorsal and 3 ventral branches in addition to terminal fork. Antennae with second segment reddish brown ; third segment brown. Frons including ocellar triangle pale brown. Orbitals in ratio of 7 : 4 : 11, anterior reclinate orbital closer to proclinate than posterior reclinate. Vibrissa strong, second oral not differentiated. Palpi pale, slender, with 3-4 marginal setae. Carina brown, broad and high. Face and cheek dark brown, greatest width of cheek 0.16 the greatest diameter of eye. Clypeus dark brown. Eyes dark red.

2.3b. *Thorax*, ♂ and ♀ : Acrostichal hairs somewhat irregular, in 8-10 rows. Prescutellars well developed. Anterior scutellars convergent ; posterior scutellars crossing each other. Anterior dorsocentral two-fifth length of posterior dorsocentral ; distance from anterior dorsocentral to posterior dorsocentral about half the distance between two anterior dorsocentrals. Mesonotum brown ; with a rectangular dark brown dorsal median patch on posterior half. Scutellum pale brown dorsal median patch on posterior half. Scutellum pale brown, with lateral sides black. Humerals two, outer thicker and long. Thoracic pleura dark brown, with a faint pale stripe. Sterno-index about 0.7. Legs yellowish brown, pre-apicals on all three tibiae ; apicals on first and second tibiae.

2.3c. *Abdomen*, ♂ and ♀ : Abdominal tergites yellow, 2T-3T with dark brown medially interrupted uniformly broad black bands, Sternites brown.

2.3d. *Wigs*, ♂ and ♀ (figure 1D) : Clear. C_1 bristle one ; C_3 bristles on basal about three-fourth of third costal section. Halteres white.

2.3e. *Periphallic organs* (figure 1A) : Epandrium yellowish brown, pubescent, broadened below, with 5 bristles on upper half and 15 closely placed bristles on lower half. Surstylus small, with 8-9 black, stout teeth arranged in a row on outer margin and a few fine setae ventrally. Cerci elongate, pubescent, with 22 small bristles.

Table 1. Average wings indices calculated from 10 ♂♂ and 4 ♀♀.

	C-index	4V-index	4C-index	5X-index
Male	2.38	2.0	1.0	2.0
Female	2.52	2.12	1.16	1.92

Average length of wing 2.78 mm (♂) ; 2.9 mm (♀)

Average length of body 2.49 mm (♂) ; 2.7 mm (♀).

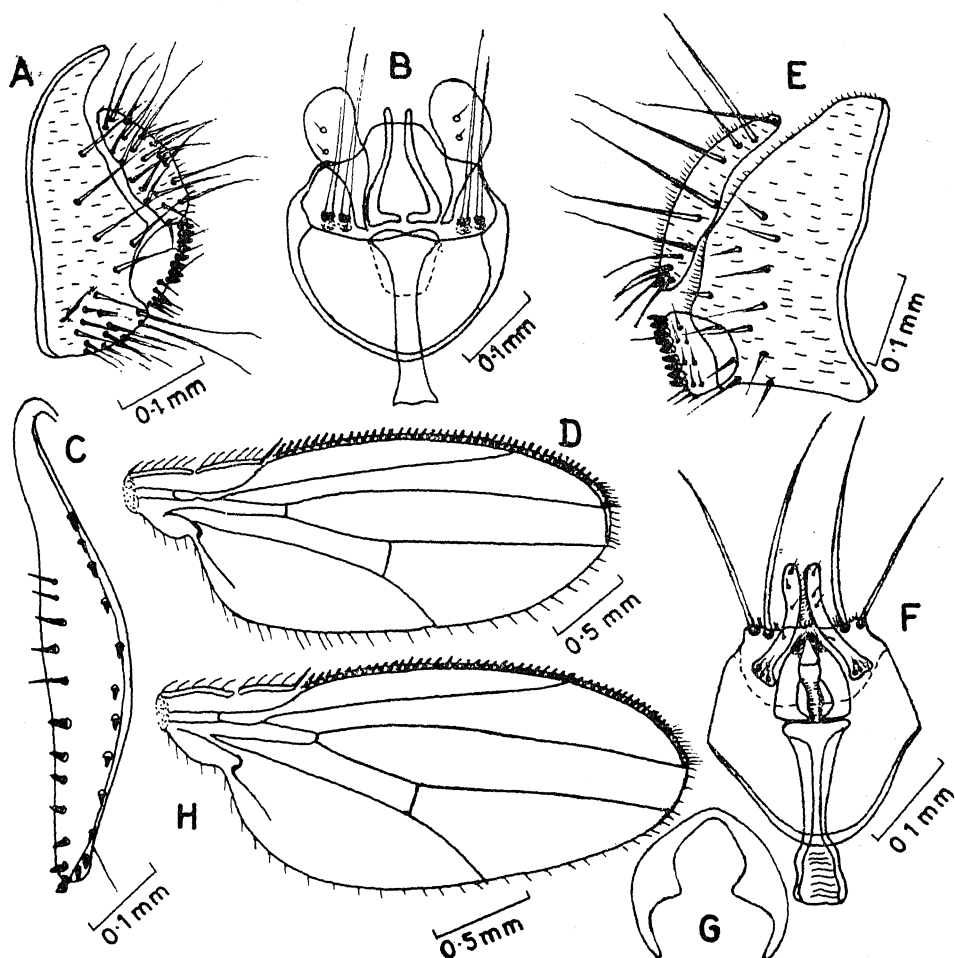


Figure 1. (A-D). *Drosophila koraputae* sp. nov.: A. Periphallic organs; sp. nov. B. Phallic organs; C. egg-guide; D. male wing. (E-H). *Drosophila neomedleri*. E. Periphallic organs; F. Phallic organs; G. Decasternum; H. Male wing.

2.3f. *Phallic organs* (figure 1B) : Aedeagus pale, bifid, broadened below, basal apodeme of aedeagus straight, about one and half times as long as aedeagus. Anterior gonapophyses pale, club shaped with 2 subapical sensilla. Hypandrium medianly projected, with two pairs of long stout submedian spines, ventral fragma hemispherical.

2.3g. *Egg-guides* (figure 1C) : Lobe yellow, elongate with 13 marginal teeth, apical tooth with broad tip, and with 11 discal teeth, upper five bristle like. Holotype ♂, India : Narayanpur, Koraput District, Orissa April 1981, Colls. Gupta and Panigrahy.

Paratypes : 7 ♂♂, 2♀♀, collection data same as holotype.

All type specimens are at present deposited in the Museum, Department of Zoology, Banaras Hindu University, Varanasi, India. 2 ♂♂ and 1 ♀ from

the paratype series are also deposited in the "*Drosophila* collection" of Department of Biology, Tokyo Metropolitan University, Tokyo, Japan.

2.3h. *Relationships* : This species in the *brunnea* group closely resembles *D. scutellimargo* Duda, but it distinctly differs from it, having a rectangular, dark brown dorsal median patch on posterior half of mesonotum (no median patch in *Scutellimargo*), 2T-3T with dark brown medially interrupted uniformly broad apical bands (2T-3T brownish yellow, with white fluorescence in *Scutellimargo*), anterior gonapophyses club shaped, with 2 subapical sensilla (large, dorsally curved with many sensilla in *Scutellimargo*), hypandrium with 2 pairs of submedian spines (1 pair in *Scutellimargo*), ventral fragma hemispherical (almost squarish in *Scutellimargo*).

Distribution : India.

2.4. *Drosophila (Scaptodrosophila) nemeodleri* : sp. nov.

2.4a. *Head*, ♂: Arista with 4 dorsal and 2 ventral branches in addition to terminal fork. Antennae with second segment reddish brown ; third segment pale brown. Frons pale brown, ocellar triangle dark brown. Orbitals in ratio of 8 : 2 : 10. Second oral thin, about half the length of vibrissa. Palpi pale brown, slender, with one prominent apical and 2-3 fine ventral setae. Carina dark brown, moderately ridged. Face and cheek dark brown, greatest width of cheek 0.14 the greatest diameter of eye. Clypeus black. Eyes dark red.

2.4b. *Thorax*, ♂: Acrostichal hairs somewhat irregular, in 6-8 rows. Anterior scutellars nearly convergent ; posterior scutellars crossing each other. Anterior dorso central half the length of posterior dorsocentral; distance from anterior dorsocentral to posterior dorsocentral about two-fifth the distance between two anterior dorsocentrals. Mesonotum and scutellum unicolorous, blackish brown, tip of scutellum white. Thoracic-pleura blackish brown. Sterno-index about 0.6. Legs brown, coxae, femora and tibia of fore legs blackish brown ; coxae and femora of mid and hindlegs dark brown ; tarsal segments of all legs yellowish brown, joints lighter. Preapicals on all three tibiae ; apicals on first and second tibiae.

2.4c. *Abdomen* ♂: 1 Tergite pale yellow, 2T with narrow medially interrupted brown apical band, 3T with medially interrupted broad band, the remainder tergites completely dark brown. Last two sternites light brown.

2.4d. *Wings*, ♂ (*figure 1H*) : Hyaline. C_1 bristle one ; C_3 bristles on basal about two-fifth of third costal section. Halteres white.

Table 2. Average wings indices calculated from 11 ♂♂.

	C-index	4V-index	4C-index	5X-index
Male	1.8	2.21	1.31	2.0

Average length of wing 2.47 mm (♂) ; Average length of body 2.74 mm (♂).

2.4e. *Peripheral organs* (figure 1E) : Epandrium brown, pubescent broadened below and narrowly projected at heel, with 12 bristles running from the middle of posterior margin downwards. Surstylus small, with 9 small dissimilar teeth arranged in a straight row on outer margin and 6 short dorso-medial and a few fine setae ventrally. Cerci brown, pubescent, narrow and elongate, with 7 upper long and 5-6 smaller bristles. Decasternum (figure 1G) brown, with lateral pieces inwardly projected.

2.4f. *Phallic organs* (figure 1F) : Aedeagus brown, short and stout, apically pointed and hairy. Basal apodeme of aedeagus straight and thick, about twice as long as aedeagus. Anterior gonapophyses pale, narrow, finger like having 4 basal sensilla and 4 equidistantly placed upper sensilla. Hypandrium with 2 pairs of strong submedian spines, inner pair little longer. Ventral fragma rounded distally.

2.4g. *Holotype* ♂, *India* : Narayanpur, Koraput District, Orissa, April, 1981
Colls. Gupta and Panigraphy.

2.4h. *Paratypes* ; 8 ♂♂, collection data same as holotype.

All type specimens are at present deposited in the Museum, Department of Zoology, Banaras Hindu University, Varanasi, India. 2 ♂♂ from the paratype series are also deposited in the "*Drosophila* Collection" of the Department of Biology, Tokyo, Metropolitan University, Tokyo, Japan.

2.4i. *Relationships* : This species resembles *D. medleri* Tsacas and Chassagnard in having somewhat similar male genital structures, but distinctly differs in having mesonotum with no silvery stripes (mesonotum with four brown broad stripes having silvery fluorescence, in *medleri*), surstylus with a row of 9 small dissimilar teeth arranged in a straight row (with a row of 11 similar strong teeth arranged in a concave row in *medleri*), ventral fragma rounded distally (almost rectangular in *medleri*).

Distribution : India.

2.5. *Drosophila* (*Scaptodrosophila*) *puriensis*, sp. nov.

2.5a. *Head*, ♂ and ♀ : Arista with 4 dorsal and 3 ventral branches in addition to long terminal fork. Antennae with second segment reddish-brown ; third segment pale brown. Frons including ocellar triangle dark brown. Orbitals in ratio of 8 : 5 : 12. Vibrissa strong, second oral not differentiated. Palpi pale brown, slender with 3-4 marginal setae. Carina brown, narrow, high and somewhat broadened below. Face and cheek brown, greatest width of cheek 0.12 the greatest diameter of eye. Clypeus dark brown. Eyes dark red.

2.5b. *Thorax*, ♂ and ♀ : Acrostichal hairs very small, in 8-10 rows between dorsocentrals. Anterior scutellars nearly parallel ; posterior scutellars crossing each other. Anterior dorsocentral half the length of posterior dorsocentral ; distance from anterior dorsocentral to posterior dorsocentral about one-third the distance between two anterior dorsocentrals. Mesonotum shiny dark brown, anteriorly lighter, with narrow and faint longitudinal streaks in the line of dorsocentrals. Scutellum blackish brown with yellowish tip. Humerals two,

subequal, outer strong. Thoracic pleura blackish-brown. Sterno-index about 0.6. Legs: Dark brown, tarsal segments slightly lighter. Preapicals on all three tibiae; apicals on first and second tibiae.

2.5c. *Abdomen, ♂ and ♀*: 1 Tergite pale yellow. 2T-4T with shiny broad black bands, the remainder tergites uniformly black. Sternites black.

2.5d. *Wings, ♂ and ♀* (figure 2D): Clear C_1 bristle one; C_3 bristles on basals three-fourth of third costal section. Halteres white.

Table 3. Average wings indices calculated from 12 ♂♂ and 14 ♀♀.

	C-index	4V-index	4C-index	5X-index
Male	2.36	2.0	1.0	1.87
Female	2.48	2.12	1.18	1.72

Average length of wing 2.52 mm (♂) 2.66 mm (♀); Average length of body 2.81 mm (♂) 3.02 mm (♀).

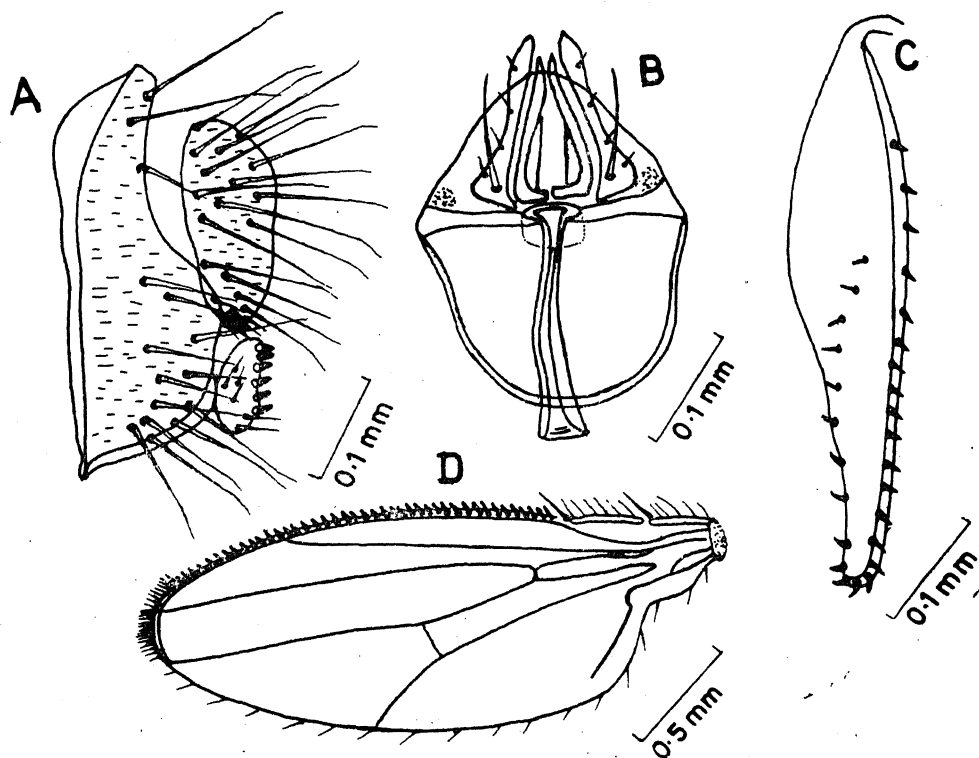


Figure 2 (A-D). *Drosophila puriensis* sp. nov. A, Peripheral organs; B, Phallic organs; C, egg-guide; D, Male wing.

2.5e. *Periphallic organs* (figure 2A) : Epandrium yellowish brown, pubescent, broadened below with 3 long bristles on upper half and 12 similar bristles on lower half. Surstylus small, with 6 small teeth arranged in a straight row and 4 dorso-medial and 2 ventral setae. Cerci pale yellow, large elongate, pubescent, with 17 long and 4-5 stout setae ventrally.

2.5f. *Phallic organs* (figure 2B) : Aedeagus pale, bifid, crescentric in lateral aspect ; narrowing distally ; basal apodeme of aedeagus about one and half times as long as aedeagus. Anterior gonapophyses pale, blade like, narrowing apically, contiguous with aedeagus, with 4 equidistantly placed marginal sensilla. Posterior gonapophyses fused together forming a triangular process. Hypandrium with a pair of submedian spines of moderate length. Ventral fragma hemispherical.

2.5g. *Egg-guides* (figure 2C) : Lobe yellow, elongate with 23 small marginal teeth and 5 discal bristles. Basal isthmus thick and short.

2.5h. *Holotype* ♂, India : Narayanpur, Koraput District, Orissa, April 1981, Colls. Gupta and Panigrahy.

2.5i. *Paratypes* : 8 ♂♂, 11 ♀♀, collection data same as holotype.

All type specimens are at present deposited in the Museum, Department of Zoology, Banaras Hindu University, Varanasi, India. 2 ♂♂ and 1 ♀ from the paratype series are also deposited in the "*Drosophila* Collection" of the Department of Biology, Tokyo Metropolitan University, Tokyo, Japan.

2.5j. *Relationships* : This species somewhat resembles *D. parabrunea* Tsacas and Chassagnand, but it distinctly differs from it in having surstylus with 6 sparsely placed stout teeth (with a group of tightly placed 12 strong teeth in *Parabrunea*), anterior gonapophyses with 4 equidistantly placed sensilla (with numerous scattered sensilla in *parabrunea*), egg-guide with 28 teeth, apical teeth placed apart (with 40 teeth, five apical teeth tightly placed in *parabrunea*), submedian spines of moderate length (usually long in *parabrunea*).

Distribution : India.

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References

- Dasmohapatra D P, Tripathy N K and Das C C 1981 Distribution of different species of *Drosophila* in Khallikote Ghats, Ganjam District, Orissa, India ; D.I.S. 56 45
Gupta J P 1972 *D. orissaensis*, A new species of *Drosophila* from Orissa, India, *Oriental Ins.* 6 561-563

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